



Asociación Latino Americana de Ecología Química  
Associação Latino Americana de Ecologia Química  
Latin American Association of Chemical Ecology



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ABSTRACT BOOK

24 - 26 NOVEMBER, 2021

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## WELCOME LETTER

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Dear Colleagues and Friends,

Bienvenidos, Bem vindos, Welcome to the 6th Congress of the Latin American Association of Chemical Ecology (VI ALAEQ). We are deeply proud to host this meeting!

Life as we know it has changed unexpectedly in 2020. The worldwide crisis of the Covid-19 pandemic forced us to reorganize our day-to-day life and work, and of course our meeting. The ALAEQ will hold a virtual congress for the first time, the only possible way to enable scientific exchange. Students and researchers will have the opportunity to expose, share and discuss their recent research. Despite the difficulties, we are very excited to announce the high involvement and participation of colleagues in all Latin America and abroad. We have 169 participants from 17 countries, including Argentina, Bolivia, Brazil, Canada, Chile, Costa Rica, Czech Republic, Denmark, Finland, France, Germany, India, Mexico, Spain, Sweden, United States, and Uruguay. We are very pleased with the participation of leading scientists from all over the world and we thank all colleagues for presenting their latest advances and research at this meeting.

In recent years, the Latin American community has suffered from limited economic resources. However, it has managed to maintain the highest scientific and academic standards, and continued to produce competitive science. From multiple perspectives and disciplines, we are gathered to examine, from chemistry to biology, the interactions between organisms and their environment: this is chemical ecology. Research presented in our meetings usually makes a great contribution to the development of sustainable and environmentally friendly pest management strategies.

We wish to thank the scientific and local organizing committees for their dedication. We extend our special thanks to the International Brain Research Organization (IBRO), The Company of Biologists, Instituto de Biodiversidad, Biología Experimental y Aplicada (IBBEA, CONICET-UBA), Departamento de Biodiversidad, Biología Experimental (DBBE, FCEN, UBA), SYNTECH, Agencia Nacional para la Promoción de la Ciencia y Tecnología (ANPCyT) and the Latin American Association Chemical Ecology (ALAEQ) for the financial support that makes possible this meeting. We also thank the Facultad de Ciencias Exactas y Naturales (FCEN, UBA) and the Instituto Nacional de Tecnología Agropecuaria (INTA) for their auspice.

The first ALAEQ meeting was in 2010 with the sole objective of building a platform for intra-regional collaborations and student exchange, and also as a bridge between Latin America and the members of the International Society of Chemical Ecology (ISCE) and the Association Asia-Pacific of Chemical Ecologists (APACE). Let's renew those ideals and have a great and productive meeting!

With the warmest regards,

Romina B. Barrozo  
General Coordinator

VI ALAEQ

## SPONSORS

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## SUPPORT ORGANIZATIONS

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## ORGANIZING COMMITTEE

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**ROMINA BARROZO**

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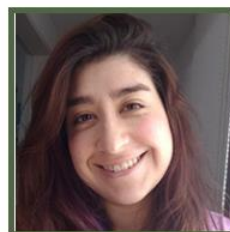
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**ISABEL ORTEGA  
INSAURRALDE**

UBA - CONICET



## SCIENTIFIC COMMITTEE

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Jorge Molina - Colombia

Andrés Gonzalez Ritzel - Uruguay

M. Fernanda G. V. Peñaflo - Brasil

Pablo Guerenstein - Argentina

Hector Masuh - Argentina

Walter Farina - Argentina

Carmen Rossini - Uruguay

Paola González Audino - Argentina

Roxana Josens - Argentina

Marcelo Lorenzo - Brasil

Jan Bergmann - Chile

## SHORT PROGRAM

(Argentine Time Zone GMT-3)

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Time zone GTM-3	DAY 1 24 - NOV	DAY 2 25 - NOV	DAY 3 26 - NOV
08:55	Opening Ceremony	Opening	Opening
09:00 - 09:40	<b>Plenary Lecture</b> Bill Hansson	<b>Plenary Lecture</b> Magali Proffit	<b>Plenary Lecture</b> Andres Gonzalez Ritzel
09:45 - 10:45	<b>Symposium</b> Molecular & Neurobiological Basis of Chemoreception	<b>Symposium</b> Anthropogenic Impact in Plant-Insect Communication	<b>Symposium</b> Semiochemicals & Pest Management in Latam
11:00 - 12:00	Oral Presentations	Oral Presentations	Oral Presentations
13:00 - 13:40	<b>Plenary Lecture</b> Norberto Peporine Lopes	<b>Plenary Lecture</b> Jeff Riffell	<b>Plenary Lecture</b> Jonathan Gershenzon
13:45 - 14:45	<b>Symposium</b> Semiochemicals Identification and Synthesis	<b>Symposium</b> Chemical Ecology & Vectors	<b>Breakout Rooms</b>
15:00 - 15:40	<b>Plenary Lecture</b> M. Carolina Blassioli Moraes	<b>Plenary Lecture</b> Cameron Currie	
15:45 - 16:45	<b>Symposium</b> Emitters & receivers: Insights in insect communication and orientation	<b>Symposium</b> Chemical Interactions Mediated by Microorganisms	<b>Closing Ceremony and ALAEQ Assembly</b>
Permanently available	Posters	Posters	Posters

Posters are permanently available during the three days

# EXTENDED PROGRAM

(Argentine Time Zone GMT-3)

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## DAY 1 – Tuesday, November 24

### 8:55 Open Ceremony

### 9:00 - 9:40 Plenary Lecture

- Bill Hansson, Max Planck Institute for Chemical Ecology, Germany - "An old and a new model for insect olfaction - flies and locusts"

### 9:45 - 10:45 Symposium Molecular & Neurobiological Bases of Chemoreception

Chair: Jose Manuel Latorre Estivalis

- Silke Sachse, Max Planck Institute for Chemical Ecology, Germany - "Elucidating olfactory processing and plasticity in the fly brain"
- Francisca Cunha Almeida, Universidad de Buenos Aires, Argentina - "Evolution of the PPK gene family in insects"
- Ben Matthews, The University of British Columbia, USA - "Salinity and surface texture cues guiding *Aedes aegypti* oviposition behaviour"

### 11:00 - 12:00 Oral Presentations

- João Pedro de Albuquerque Souza, Universidade Federal do Paraná, Brazil - "Enantioselective synthesis of methyl-branched cuticular hydrocarbon (r)-(-)- and (s)-(+)-3-methylhenicosane"
- Milena Manzur, IIBIO-CONICET-UNSAM, Argentina - "Domesticated vs native vegetation: variation in voc emissions among solanum genus plants"
- Rocío Lajad, Universidad de Buenos Aires, Argentina – "Foraging preferences in honeybees after experiencing adulterated pollen"
- Callum Kingwell, Cornell University, USA – "Queen pheromones advertise the benefits of cooperation in a flexibly social bee"
- Ricardo Godoy, Universidad de La Frontera, Chile – "Molecular and functional characterization of aldehyde oxidases from the great wax moth, *Galleria mellonella* as odorant degrading enzymes"

### 12:00 - 13:00 Lunch Break

### 13:00 - 13:40 Plenary Lecture

- Norberto Peporine Lopes, University of São Paulo, Brazil - "Role of Small Molecules in Ecological Interactions"

**13:45 - 14:45 Symposium Semiochemicals Identification and Synthesis**

Chair: Diogo Vidal

- Moacir Rossi Forim, UFSCar, Brazil - "Same Species, Different Chemical Profiling: Genotype-environment interaction seeking bioactive molecules"
- Jocelyn Millar, UC Riverside, USA - "Click chemistry: identification of sex pheromones for North American click beetle species (Elateridae)"
- Daiane Szczerbowski, UFMG, Brazil - "Synthesis and identification of unusual terpenoid esters isolated from the androconia of *Heliconius erato*"

**15:00 - 15:40 Plenary Lecture**

- M. Carolina Blassioli Moraes - Universidade de São Paulo, Brazil - "EMBRAPA contributions to pheromone studies of neotropical insects-pests and their application to pest management"

**15:45 - 16:45 Symposium Emitters and Receivers: Insights in Insect Communication and Orientation**

Chairs: Raul Laumann and Walter Farina

- Christian Sherley Araujo da Silva Torres, Universidade Federal Rural de Pernambuco, Brazil - "Semiochemicals of Coccidophagous lady beetles affect their behavior and development"
- Santiago Ramírez, University of California, Davis, USA - "The evolution of chemical communication and speciation in orchid bees"
- Roxana Josens, University of Buenos Aires, CONICET, Argentina - "Pheromonal modulation of behavior in ants"



## DAY 2 – Wednesday, November 25

### **9:00 - 9:40 Plenary Lecture**

- Magali Proffit, Centre d'Ecologie Fonctionnelle et Evolutive, France - "Effect of air pollution on plant-pollinator chemical communication"

### **9:45 - 10:45 Symposium Anthropogenic impact in Plant-Insect Communication**

Chairs: Carmen Rossini and Jorge Zavala

- Maria Victoria Coll Araoz, PROIMI-CONICET, Argentina - "The effect of domestication on the incidence of maize viral and stunting diseases"
- Shannon Olsson, National Center for Biological Sciences, India - "The impact of air pollution on pollinators"
- James Blande, University of Eastern Finland, Finland - "Volatile-mediated interactions in polluted environments: consequences for chemical communication in conifers"

### **11:00 - 12:00 Oral Presentations**

- Katherine D. Mosquera, René Rachou Institute, Brazil - "Oviposition responses of *Aedes aegypti* and identification of volatiles from mosquito-associated symbiotic bacteria pathogenic fungus modulates the insect-plant interaction favoring its infection and dissemination"
- Vicente Machado, Universidade Estadual Paulista "Júlio de Mesquita Filho", Brazil - "Evaluation of a synthetic human odour blend for the attraction of the sand fly *Lutzomyia longipalpis*"
- Amanda Túler, Universidade de São Paulo, Brazil - "Pathogenic fungus modulates the insect-plant interaction favoring its infection and dissemination"
- Teruyuki Matsunaga, University of Berkeley, USA - "Evolution of olfactory receptors tuned to mustard oils in herbivorous *Drosophilidae*"
- Patricia C Fernández, CIHIDECAR-CONICET, Argentina – "Dramatic effects of an invasive outbreak species on fitness of a native herbivorous species is indirectly mediated by plants"

### **12:00 - 13:00 Lunch Break**

### **13:00 - 13:40 Plenary Lecture**

- Jeff Riffel, University of Washington, USA - "The sensory bases of nectar-seeking by mosquitoes"

**13:45 - 14:45 Symposium Chemical Ecology & Vectors**

Chairs: Ligia Borges and Pablo Guerenstein

- Zainulabeuddin Syed, University of Kentucky, USA- "Molecular signatures of sand fly communication"
- Tiago Feitosa Mota, FIOCRUZ, Brazil - "Identification of bioactive compounds against medically important arthropod vectors by bioassays and in silico assays"
- Lucia Ibarra Bouzada, CICyTTP, Argentina - "Trapping of triatomines, Chagas disease vectors, in experimental boxes that mimic an insect natural habitat"

**15:00 - 15:40 Plenary Lecture**

- Cameron Currie, University of Wisconsin-Madison, USA - "Ants, agriculture and antibiotics"

**15:45 - 16:45 Symposium Chemical Interactions Mediated by Microorganisms**

Chairs: Monica Puppo and Marcelo Lorenzo

- Ainhoa Martínez Medina, IRNASA-CSIC, Spain - "Root mutualistic fungi modulate plant-herbivore interactions at multitrophic level"
- Laura V. Flórez, University of Copenhagen, Denmark - "Recruiting and maintaining a symbiotic community that defends a beetle against fungal pathogens"
- Taicia Fill, UNICAMP, Brazil - "The chemistry hidden in the biological interactions found in the citrus host"

## DAY 3 – Tuesday, November 26

### 9:00 - 9:40 Plenary Lecture

- Andres González Ritzel, Universidad de la República - "The chemical ecology of insect pests in Uruguay, similar questions in diverse study systems"

### 9:45 - 10:45 Symposium Semiochemical & Pest Management in Latam

Chairs: Paola Gonzalez Audino and Hector Masuh

- Francisco Gonzalez, ChemTica Internacional S.A., Argentina - "Uso de semioquímicos para control de plagas en América Latina desde la perspectiva comercial"
- Enrique Lobos, Facultad de Agronomía y Agroindustrias, Argentina - "Posibilidades de incorporación de los semioquímicos en el manejo de plagas en cultivos de Argentina"
- Jeremy Allison, Canadian Forestry Service, Canada - "Applied Chemical Ecology and Trap Development: Can we move beyond Origami?"

### 11:00 - 12:00 Oral Presentations

- Silvina Belliard, Instituto Nacional de Tecnología Agropecuaria, Argentina - "Identification of guava volatile compounds capable of stimulating *Anastrepha fraterculus* male sexual competitiveness"
- Guillermo Reherrmann, Swedish University of Agricultural Sciences, Sweden - "Behavioral manipulation of *Drosophila suzukii* by yeast volatiles for pest control"
- Pilar Altamar-Varón, Universidad Militar Nueva Granada, Colombia - "Advances on describing the *Copitarsia uncinata* sex communication. where are we now?"
- Virginia Usseglio, Instituto Multidisciplinario de Biología Vegetal, Argentina - "Effects of maize grain epicuticle on grain-insect-fungus interaction"
- Daniel Torrico Bazoberry, Facultad de Ciencias, Universidad de Chile, Santiago, Chile - "Chemical responses of tobacco plants induced by vibrational signals of a generalist herbivore"

### 12:00 - 13:00 Lunch Break

### 13:00 - 13:40 Plenary Lecture

- Jonathan Gershenzon, Max Planck Institute for Chemical Ecology, Germany - "Finding friends in the forest: Volatiles of bark beetle-associated fungi help maintain symbiosis with beetles"

### 13:45 - 15:40 Breakout Rooms

### 15:45 - 16:45 Closing Ceremony and ALAEQ Assembly

## ABSTRACTS

(Expositors' alphabetical order)

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### THE STUDY OF CHEMICAL COMMUNICATION IN HEAD LICE AS CONTROL STRATEGY AGAINST PEDICULOSIS

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Poster / Insect communication

*Pediculus humanus capitis* is an obligate bloodsucking ectoparasite of the human scalp. Pediculosis affects mainly kids at scholar age. Chemical cues are involved in the head lice - human interaction. Recent studies of our laboratory demonstrated that adult head lice responded to host chemical stimuli. Moreover, these studies demonstrated that head lice were highly attracted to volatiles present in human scalp, they were identified and attraction was determinate towards the major individual components nonanal, sulcatone, geranyl-acetone and palmitic acid. More recently, we demonstrated that head lice recognize and prefer the whole odor (highly and moderately volatile emanations) of the human head over the whole odor of other parts of the human body. In this work, we studied the head louse attraction produced by the mixture of major volatiles from human scalp extracts compared to their isolated components. So, we evaluated the head louse attraction produced by human scalp main components alone and in mixtures. Although volatile fraction composition was already available from Galassi *et al.* 2018, we identified the whole odor (highly and moderately volatile emanations) composition in order to select major components for individual and in mixtures evaluation. We tried to establish if there are critical compounds involved in the attraction behavior and if there is an optimal mixture involving attraction behavior of lice towards the human host. Volatile blends of nonanal, sulcatone and geranylacetone presented a synergistic attractive effect ( $0,10 > p\text{-value} > 0,05$ ) compared against isolated compounds. Whole odor tests are still in process.

## **APPLIED CHEMICAL ECOLOGY AND TRAP DEVELOPMENT: CAN WE MOVE BEYOND ORIGAMI?**

**Allison J**

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Canadian Forestry Service, Canada.

Symposium / Semiochemical & Pest Management in Latam

Studies have demonstrated that semiochemical-baited intercept traps differ in their performance for sampling forest insects, but we have an incomplete understanding of how and why intercept trap design effects vary among species and habitats. This can significantly delay both the development of new and optimization of existing survey and detection tools. The development of a mechanistic understanding of why trap performance varies within and among species would mitigate this delay. This presentation will provide an overview of factors known to affect the performance of intercept traps for forest Coleoptera and field experiments designed to identify mechanisms driving these effects. Field trials have demonstrated that the number of Cerambycidae approaching to within 3 m downwind does not differ among intercept trap designs but differences in the number of beetles contacting the trap were observed. Studies with carbon dioxide observed differences in plume structure downwind of these same trap designs but the differences were not consistent with differences in trap performance. Field trials that manipulated visual stimuli associated with intercept traps found support for the hypothesis that differences in visual stimuli associated with different trap designs contributes to differences in performance among taxa.

## EVOLUTION OF THE PPK GENE FAMILY IN INSECTS

Latorre-Estivalis JM<sup>1</sup>, Almeida FC<sup>2</sup>, Pontes G<sup>3</sup>, Dopazo H<sup>4</sup>, Barrozo RB<sup>5</sup>, Lorenzo MG<sup>6</sup>

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Symposium / Molecular & Neurobiological Bases of Chemoreception

Insect pickpocket (PPK) receptors mediate diverse functions, among them the detection of mechano- and chemo-sensory stimuli. These receptors are encoded by genes belonging to a single gene family in insects. To have a better understanding of their evolution we searched for PPK genes in the genomes of 26 species of 8 orders including holometabolous and hemimetabolous insects (Blattodea, Orthoptera, Hemiptera, Phthiraptera, Hymenoptera, Lepidoptera, Coleoptera, and Diptera). Our searches found 578 PPK genes in total and at least one gene per species. Phylogenetic analyses support their classification into 7 subfamilies, of which, subfamily VII, represented by *ppk17* in *Drosophila melanogaster*, was the most divergent. The PPK gene family evolved according to a gene birth-and-death model that generated lineage-specific expansions, which were usually located in clusters in the genome. The different subfamilies had very different number of genes and evolutionary dynamics: some were more conserved across insect orders whereas other subfamilies had high turnover rates. Significant differences in repertoire were observed among species: on one hand, *Musca domestica* (59), *Aedes albopictus* (51), *Culex quinquefasciatus* (48), and *Blattella germanica* (41) presented the largest PPK repertoires and on the other hand, *Pediculus humanus* (only *ppk17*), bees and ants (6-9) had the smallest PPK sets. A subset of prevalent PPKs was identified, indicating very conserved functions for these receptors. Finally, at least twenty percent of the sequences presented calmodulin-binding motifs, suggesting that these PPKs may amplify sensory responses similarly as proposed for *D. melanogaster ppk25*. Overall, this work characterized the evolutionary history of these receptors revealing relevant unknown gene sequence features and clade-specific expansions.

## ADVANCES ON DESCRIBING THE *Copitarsia uncilata* SEX COMMUNICATION. WHERE ARE WE NOW?

Altamar-Varón P<sup>1,2</sup>, Rodríguez D<sup>1</sup>, Coy-Barrera E<sup>2</sup>  
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Oral / Semiochemicals and pest management

*Copitarsia uncilata* was described in 2010 by Burgos & Leyva as an ornamental crop pest and, in Colombia, it is a genuine problem to this productive sector. Sex pheromones are increasingly used as a promising management tool for monitoring and controlling lepidopterans including *Copitarsia* species. In the case of *C. uncilata*, we have performed several steps to develop a behavioral-based management method. Initially, their distribution in Bogotá plateau municipalities was found to be mainly hosted by *Alstroemeria* sp. crops. A careful inspection of the genitalia, mostly by the uncus shape, led to the morphological taxonomy recognition. Subsequently, the life cycle of *C. uncilata* was described and an artificial diet-based rearing was also developed. The reproductive behavior (particularly age and moment for calling and mating) was then determined. *C. uncilata* females perform the calling behavior 2 days after emergence and during the first third of the scotophase. The chemical composition of the pheromone gland was studied; and five pheromone type 1-like compounds and several composition differences with *C. decolora* were detected. Wind tunnel essays confirmed this difference, since *C. uncilata* male adults had a low source landing ( $\approx 14\%$ ) when exposed to the *C. decolora* commercial pheromone blend. The highest values of oriented flights, landing and copulation attempts ( $>89\%$ ), occurred mainly when conspecific females and bioactive extracts were evaluated. Consequently, some capturing techniques were employed to determine the female's effective emission and to identify the *Copitarsia*'s sex attraction-related compounds. Simultaneously, in-silico analysis was performed using a homologous noctuid OBP (odorant binding protein) to rank several *Copitarsia*-reported pheromone compounds by affinity. These studies provided key information aiming at integrating semiochemicals within IPM in *Alstroemeria* crops. In this regard, further studies are currently underway to complement and definitively define the conspecific communication related compounds and its later use in baited traps.

## CHANGES IN THE STRUCTURE OF POLYSACCHARIDES FROM HULL CELL WALLS PRODUCED BY ATTACK OF STINKBUG *Nezara viridula* L. ON FIELD DEVELOPING SOYBEAN SEEDS

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Poster / Plants-organisms interactions

*Nezara viridula* is a pest in soybean crops. Soybean responds to stinkbug attack inducing defenses regulated by pathways of salicylic acid and jasmonic acid. Cell walls can be an effective barrier against piercing-sucking insects. No detailed study on hull polysaccharides in the immature stage, when *N. viridula* attack takes place has been reported. The aim of this work was to determine if stinkbug damage produces changes in cell wall polysaccharides. Hull cell wall polysaccharides from seeds of a commercial cultivar, Williams, were studied. One untreated (CTR) lot and two different treatments were prepared, during R5 stage: (1) stinkbug damaged – insects were placed on pods and enclosed with a fine mesh bag, during 15 days (BD); (2) pods sprayed with 1.5 mM of salicylic acid every 4 days, during 15 days (TSA). Collection was done when seeds were in R6 stage. Sequential extraction with water, 0.05 M CDTA (pH 6), 0.05 M Na<sub>2</sub>CO<sub>3</sub>, 1M KOH, and 4M KOH was carried out. The Na<sub>2</sub>CO<sub>3</sub> extract was fractionated by anion exchange chromatography on DEAE-Sephadex A-25. The major fraction (F2) eluted with 0.5 M NaCl, the monosaccharide composition was determined by GC-MS, the total carbohydrates and uronic acids were determined colorimetrically, and the structures were analyzed by NMR. Important differences were observed in the neutral monosaccharide composition. The amount of Rha was important in these extracts, indicating major quantities of rhamnogalacturonans, in particular, a 50% decrease was observed in TSA vs. CTR and BD. While the Man content in BD decreases almost 50% with respect to CTR and even more with respect to TSA, indicating minor quantities of galactomannans. This analysis proves that, because of stinkbug attack, changes occur in cell walls, which can be measured using polysaccharide chemical analysis, and not only by differences in the expression of genes related to cell wall biosynthesis.



## CHEMOSENSORY RECEPTION IN THE STINGLESS BEE *Tetragonisca angustula*

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Poster / Insect communication

The relationship between chemosensory abilities and task division has been poorly studied in stingless bees. Thus, we examined odor reception and sugar responsiveness of the social stingless bee *Tetragonisca angustula*, focusing on workers involved in different tasks. Using the proboscis extension response, we evaluated sucrose response thresholds (SRTs) of foragers and guards. Additionally, we studied the response thresholds of foragers to glucose and fructose. Peripheral responses to odors at the antennae of foragers and guards were recorded by electroantennography (EAG). Besides, we quantified and described the number and type of sensilla present on the antennae of those bee groups using scanning electron microscopy. We found that foragers' SRTs changed according to the resource collected: non pollen foragers (NPF) showed higher SRTs than pollen foragers (PF) and guards, which showed similar sucrose responsiveness. Also, pollen foragers showed different response thresholds according to the type of sugar offered (sucrose > glucose > fructose), while both NPF and PF presented a lower response to fructose compared to sucrose and glucose. EAG signal strength of both foragers and guards increased with increasing odor concentration. Interestingly, guard bees showed the highest response to citral, an odor that triggers defensive behavior in *T. angustula*. The type and number of sensilla present in the antennae of guards and foragers were similar. These results suggest that differences found in chemosensory responses among worker subcastes are task dependent and might be involved in regulating the division of labor.

## LOCAL AND SYSTEMIC SOYBEAN RESPONSE TO *Nezara viridula* L.

**Barriga LG**<sup>1</sup>, Manetti M<sup>2,3</sup>, Chludil H<sup>1</sup>, Zavala J<sup>2,3</sup>

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Poster / Plants-organisms interactions

Soybean (*Glycine max* (L.) Merr.) is the most widely spread crop over Argentinian soil. Higher economic returns are achieved with chemical control of insect pests, such as the stink bug *Nezara viridula* L. Nymphs and adults feed on developing and mature seeds, piercing with their stylets and reducing weight, quality, and total number of seeds per plant. There are natural chemical responses that are induced in plants against herbivore attack, in damaged and/or undamaged tissues. Phenolic compounds such as isoflavonoids are secondary metabolites associated with soybean response and resistance to insect herbivory. Deep understanding of plant response would allow breeding programs to identify and enhance genetic alternatives to reduce pesticides dependency. Under field conditions, we studied the soybean cultivar Williams response (isoflavonoids diversity and concentration) in pods that received only one insertion of *Nezara viridula* L. mouthparts, for 30 minutes. This chemical response was analyzed in the pierced seed (local effect) as well as those seeds, from the same pod, that did not receive herbivory (systemic effect). Isoflavonoid identity and quantification were evaluated through a methanol extraction followed by HPLC-UV. Our results show that developing seeds present at least five constitutive glucoside isoflavonoids. Cultivar Williams induces all isoflavonoids up to 72h after only one insertion of *Nezara viridula* L. mouthparts. Genistein malonyl glucoside and Daidzein malonyl glucoside are also induced as a systemic response. Future studies should be carried out to confirm if any of these isoflavonoids have direct negative effects over *Nezara viridula* L. biology and what are the effective concentrations in which these mechanisms occur.

## PHENOLIC COMPOUNDS AS BIOPESTICIDES FOR *Sitophilus zeamais*

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Poster / Semiochemicals and pest management

The insect *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), known as the maize weevil, produces significant economic losses during the storage of these grains in Argentina. The damage caused by *S. zeamais* infestations is usually of two types: direct damage, which is caused by the consumption of the grain, and indirect damage caused by secondary pests such as bacteria and fungi. In the silo infestation, the insect's olfactory perception of the VOCs acquires great importance since it influences on the most important behavior patterns, such as the search for food, partner and oviposition site.

Based on this, our objective was to evaluate the effect of 10 natural phenolic compounds on the behavior and survival of *S. zeamais*. For this, a repellent/attraction test, using a two-ways olfactometer, and a contact toxicity assay were carried on. In this study we observed that the thymol, phenol and p-cymene present a repellent effect on *S. zeamais* while the guaiacol, m-cresol and vanillin were attractive to this insect. On the other hand, the o-cresol and thymol present the highest insecticidal activity. In relation with this, structural and physicochemical properties of the chemical compounds, such as the pKa, number and position of -OH groups and the chirality of principal carbon, among others, are related with its bioactivity and efficacy.

Traditionally, the control of *S. zeamais* is carried out using synthetic pesticides, which cause several damages in the environment and human health. For this reason, the study of the bioactivity of natural compounds to ensure food safety has become of great importance.

## IDENTIFICATION OF GUAVA VOLATILE COMPOUNDS CAPABLE OF STIMULATING *Anastrepha fraterculus* MALE SEXUAL COMPETITIVENESS

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Oral / Semiochemicals and pest management

In some tephritid fruit flies, exposure to volatile compounds from some plants increase male sexual success. This phenomenon has been used to boost sterile males' sexual competitiveness in the framework of the sterile insect technique (SIT). Previous studies revealed that males of *Anastrepha fraterculus* (Diptera: Tephritidae) exposed to volatiles from guava (*Psidium guava*) fruit (GF) and guava essential oil (GEO) exhibit intensified courtship behavior and have greater copulatory success relative to unexposed males. Similar results were achieved through Huingán (*Schinus polygamus*) essential oil or lemon (*Citrus limon*) essential oil exposure. To identify the responsible compounds involved in these effects, we compared the volatile chemical profiles of GF, GEO, Huingán essential oil and lemon essential oil. We identified five common compounds: (E) - $\beta$ -Ocimene, (Z) - $\beta$ -Ocimene, Limonene,  $\beta$ -Caryophyllene and  $\alpha$ -Humulene. We used the electroantennographic detection (EAD) technique to verify that males were able to detect all the identified compounds and to build dose-response curves between 0.01  $\mu\text{g}/\mu\text{l}$  and 100  $\mu\text{g}/\mu\text{l}$  for each compound. Furthermore, we confirmed a stimulating effect on the courtship behavior of males for each of these compounds, which were assayed on a wide dose range of exposures (1-100  $\mu\text{g}/\mu\text{l}$ ). However, we found that the concentration that maximized the effect on courtship behavior differed among compounds by several orders of magnitude. Our results may contribute to the ongoing development of the SIT in this species.

## EXTRACTION AND IDENTIFICATION OF VOLATILES ORGANIC COMPOUNDS (VOCs) FROM IN VITRO INFECTION OF *Leishmania* (*Leishmania*) *amazonensis* (KINETOPLASTIDA:TRYPANOSOMATIDAE)

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Poster / Identification and synthesis of semiochemicals

Pathogenic parasites can modify the production of volatile organic compounds (VOCs) emitted by host and affect the attractiveness of vectors to them, as demonstrated for *Plasmodium* spp. and *Leishmania infantum*. For *L. amazonensis*, there are no investigations on such phenomena. This study aimed to evaluate possible differences in the VOCs released by host macrophages, uninfected or infected by *L. amazonensis* in culture medium. In a first step, fully differentiated macrophages were obtained from BALB/c mice bone marrow and then  $1.5 \times 10^6$  macrophages/well were placed into two six well plates. One plate remained uninfected and on the other plate the macrophages were infected with *L. amazonensis* PH8 strain (MOI=10:1; 24 hours). In a third plate, promastigotes of *L. amazonensis* were incubated in culture medium with pH of 5.2 for their differentiation in amastigotes. Each one of the plates had their volatiles extracted individually with a solid phase microextraction (SPME) fiber during a period of 24 hours. After the extraction, the SPME fibers were injected for desorption of volatiles and analysis by gas chromatography-mass spectrometer. From the chromatograms obtained for the samples of uninfected macrophages, infected macrophages and amastigotes, it was observed a total peak number of 87, 84 and 83, respectively. After retention index calculation and further identification of these peaks, we could identify a total of 53 volatiles compounds, of which 12 were identified exclusively in the amastigotes culture and other 5 exclusively in the infected macrophages. The relative areas of the identified compounds were then submitted to a Principal Component Analysis and a cluster analysis that showed a correlation of groups of compounds with two samples: amastigotes and macrophages, regardless of the infection. Altogether our results suggest that *L. amazonensis* infection in vitro can release a specific pattern of VOCs.

## DEVELOPMENT OF MONITORING AND CONTROL USING THE SEX PHEROMONE OF *Pseudococcus calceolariae* (HEMIPTERA: PSEUDOCOCCIDAE) IN FRUIT ORCHARDS IN CHILE

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Poster / Semiochemicals and pest management

The citrophilus mealybug *Pseudococcus calceolariae* (Maskell) is an economically important pest in fruit orchards in many regions of the world. The recent identification of its sex pheromone allows the development of applications for its use in integrated pest management programs. We carried out field experiments to evaluate the potential use of the pheromone in monitoring and control via mating disruption. For monitoring, we evaluated the effect of the isomeric purity of the synthetic pheromone, the pheromone dose, and the age of septa exposed to field conditions on catches of males in pheromone-baited Delta traps. We also examined the correlation of trap captures with abundance of females on plants (during the growing period) and fruits (at harvest). The potential of mating disruption was evaluated during two non-consecutive seasons in tangerine and apple orchards, where a reduction of male captures in pheromone traps was observed after deployment of the pheromone in a polymeric matrix. Due to low abundance of mealybugs on the trees, we did not observe a reduction of populations or damage.

## VOLATILE-MEDIATED INTERACTIONS IN POLLUTED ENVIRONMENTS: CONSEQUENCES FOR CHEMICAL COMMUNICATION IN CONIFERS

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Symposium / Anthropogenic Impact in Plant-Insect Communication

Plants interact with other members of their community, including neighbouring plants, through the emission of volatile organic compounds. In our recent research, we have observed clear indications that feeding by the large pine weevil, *Hylobius abietis*, induces volatile emissions that mediate interactions between pine seedlings. To our knowledge this is the first observation of volatile-mediated plant-plant interactions in a coniferous species. Over the course of the last decade, data showing a negative effect of air pollution on volatile-mediated interactions has accumulated. The secondary pollutant, tropospheric ozone, can affect multiple parts of the chemical messaging process, resulting in elimination or alteration of the message reaching a recipient. We investigated the effect of ozone pollution on the volatile emissions of herbivore-damaged emitter plants and on the responses of receiver plants. In this talk we will present new data supporting the presence of volatile-mediated interactions in Scots pine and a negative effect of ozone pollution on those interactions. Our new findings are based principally on combining plant ecophysiology and chemical ecology.

## EMBRAPA CONTRIBUTIONS TO PHEROMONE STUDIES OF NEOTROPICAL INSECTS-PESTS AND THEIR APPLICATION TO PEST MANAGEMENT.

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Plenary

In Brazil and other countries with huge areas with non-tillage crops systems, agriculture production is harmed by constant insect outbreaks, requiring higher use of insecticides, raising new and old worries about the massive use of toxic compounds in the environment. In the last 30 years, the research based on natural products and semiochemicals in Brazil has increased significantly, bringing other possibilities to manage agriculture pests. This talk intends to present the Embrapa contribution to this field, covering the basic studies on pheromone identification and the steps to transform the pheromone into a useful tool for growers. The first pheromone studied at EMBRAPA in the 1980s was the sex pheromone of a Brazilian population of *Nezara viridula*, an important grain pest in Brazil. Subsequently, another important soybean pest, the brown stink bug *Euschistus heros*, had its pheromone identified as methyl 2,6,10-trimethyltridecanoate, with the stereochemistry elucidated as 2*R*,6*S*,10*S* through behavioural bioassays using all eight synthetic stereoisomers. Other soybean and rice stink bugs also had their pheromone identified, such as: the soybean pests *Piezodorus guildinii*, *Thyanta perditor*, *Chinavia ubica* and *C. impicticornis*, and the rice pests *Tibraca limbativentris* and *Oebalus poecilus*. In 2006, the pheromone of the rice stink bugs *Tibraca limbativentris* was identified as two isomers of 1,10-bisbolen-3-ols (zingiberenol), and recently, using two different chiral columns, the absolute configuration of these molecules was elucidated as (3*S*,6*S*,7*R*) and (3*R*,6*S*,7*R*). The aggregation pheromone of the lesser mealworm, *Alphitobius diaperinus*, was identified as a six-component blend emitted only by males: (*R*)-limonene, 2- nonanone, (*E*)-ocimene, (*S*)-linalool, (*R*)-daucene, and (*E,E*)- $\alpha$ -farnesene. Behaviour studies with this blend showed that only the isomer produced by the males was active for attraction. The efforts to identify these and others pheromones will be presented, and the chemical ecology involved and possible applications will be discussed.



## EFFECT OF THE EXPOSURE TO *Humulus lupulus* ESSENTIAL OILS ON THE CUTICULAR HYDROCARBONS PROFILES OF HONEYBEES

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Poster / Identification and synthesis of semiochemicals

The animal pollination has an important role on regulation of agroecological services: about 90% of flower plants depend on animals for pollen transportation. *Apis mellifera* (Hymenoptera: Apidae), currently one of the most important pollinators, has lately decreased their global quantity. Among the factors influencing this decrease, the most important ones are the use of agrochemicals and the incidence of the mite *Varroa destructor* (Mesostigmata: Varroidae). The use of natural miticides based on essential oils (EO) has been largely evaluated as an alternative means to control varroa; however, sublethal effects of EO have been scarcely studied. In this work, we characterized the EO from four varieties (Cascade, Nugget, Styrian-Golding and Fuggle) of *Humulus lupulus* (Cannabaceae) using GC-MS; evaluated the toxicity of two EO (Cascade and Nugget) on mites and honeybees using a total exposure method; and finally assessed the effect of the EO exposure on cuticular hydrocarbon (CHC) profiles of honeybees as a proxy for detection of sub-lethal effects. The main components of the essential oils were  $\beta$ -myrcene,  $\beta$ -caryophyllene,  $\beta$ -farnesene,  $\alpha$ -humulene,  $\alpha$ -selinene,  $\gamma$ -cadinene,  $\delta$ -cadinene and humulene-epoxide II. The Cascade EO has a security index of 3.8 ( $LC_{50\ 24h, \text{mites}}$  of 1.61  $\mu\text{L/mL}$  (CI95%: 1.19-2.06  $\mu\text{L/mL}$ );  $LC_{50\ 24h, \text{honeybees}}$  of 6.10  $\mu\text{L/mL}$  (CI95%: 5.22-7.18  $\mu\text{L/mL}$ )), which is considered safe for its use on honeybees. For the CHC, no effects of the EO exposure were detected (compared with the solvent) on compound groups (ANOVA with post-hoc Dunnett's test,  $p > 0.05$  for all). However, some individuals CHC (ANOVA with post-hoc Dunnett's test,  $p < 0.05$ ) were affected by the exposure to the Nugget EO at 1.4 and 2.8  $\mu\text{L/mL}$  dose and Cascade EO at 2.8  $\mu\text{L/mL}$ , being n-nonadecane, n-heneicosane, n-nonacosane, n-hentriacontane and 7-nonacosene the most affected ones. These results highlight the importance of considering sublethal effects when developing new control products.

## INCIDENCE OF FOLIAR TRICHOMES ON THE OVIPOSITION OF *Epilachna paenulata*

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Poster / Plants-organisms interactions

Herbivorous insects use chemical and physical characteristics of their hosts to recognize the best ones to complete their cycle. Trichomes, as physical and chemical barriers, prevent insect feeding and oviposition. *Epilachna paenulata* (Coleoptera: Coccinellidae) can complete its cycle in different Cucurbitaceae plants, including *Cucurbita maxima* (CMA) and *Cucurbita moschata* (CMO). Nevertheless, we here show that females: a) prefer to lay eggs; b) lay more eggs; and c) live longer on CMA (regardless of their diet when larvae; GLM,  $p < 0.01$  for all cases). To determine plant cues mediating this female oviposition preference, we further studied the chemical and trichome variability between these 2 species. CMA and CMO were compared in their profile of volatile compounds, epicuticular waxes and fixed compounds of the parenchyma (multivariate analysis) and could be distinguished by their chemistry. However, both species provide the same conditions for larval development (GLM,  $p > 0.05$  for: Development Time, Mass/Stage and Total Mass Acquired) so the preference for CMA would not respond to the preference–performance hypothesis. The foliar trichomes were characterized and quantified, identifying 4 types of trichomes: 2 glandular (capitate and multicellular) and 2 tectors (short and long). The number of trichomes was higher in CMO plants, except for capitate glandular trichomes. The short-tector trichomes were the most abundant, uniformly distributed on both sides of the leaves in CMO and in greater abundance on the abaxial surface of CMA. We hypothesize that oviposition preference of *E. paenulata* for CMA could negatively correlate to the larger number of trichomes in CMO leaves. Results suggest that females choose the plant that maximizes their own fitness, as is reflected in the greater longevity and fertility achieved in CMA (GLM,  $p < 0.01$  for all variables studied). Chemical and trichome variability are discussed in their role to determine oviposition preference of *E. paenulata*.

## RESPONSE OF *Anastrepha fraterculus* WIEDEMANN (DIPTERA: TEPHRITIDAE) TO SYNTHETIC SEMIOCHEMICALS

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Poster / Semiochemicals and pest management

*Anastrepha fraterculus* is an important fruit pest in South America. Synthetic semiochemicals are not available to monitor its presence in the field. This study was conducted to determine the attractiveness of three synthetic semiochemicals. Attraction was tested on mature copulated males and females, and mature virgin females (10 to 14 days old) from a laboratory strain. The semiochemicals evaluated were epianastrephin (EAG+), anastrephin (EAG-) (70:30 and 95:5) and a synthetic epianastrephin analog with two methyl groups (dimethyl). A natural pheromone release unit (10 sexually mature males) was used as positive control and water as negative control. One hundred flies of a given sex or mating condition were released in cylindrical field cages (3 m diameter x 2 m high) under natural conditions. Attractants were placed in McPhail traps (one trap with attractant and one trap with water per cage). Ten repetitions were performed per treatment. The number of flies captured in the trap with attractant was compared to the control trap (water) within each treatment (semiochemical, sex and mating condition in females) by means of a t test for paired samples (one-tail). A GLM was used to compare attractants. Mated males were significantly more attracted to dimethyl and epianastrephin 70:30 than to the control ( $P=0.0134$  and  $P=0.0500$ , respectively). Mated females were significantly more attracted to the lek than to the control ( $P=0.0348$ ). Virgin females were significantly more attracted to epianastrephin 70:30, epianastrephin 95:5 and the lek than to the control ( $P=0.0413$ ,  $P=0.0288$  and  $P=0.0231$ ). Results from the GLM showed that the highest attraction occurred for epianastrephin 70:30 in virgin females. Our bioassays performed under semi-field conditions showed that traps baited with synthetic semiochemicals are attractive to *A. fraterculus* males and females. Following validation under open field conditions, these specific semiochemicals could be used for pest monitoring.

## **DROUGHT STRESS AND PLANT GROWTH-PROMOTING RHIZOBACTERIA: THIS INTERACTION INFLUENCE THE TOTAL ESSENTIAL OIL YIELD, IT'S COMPOSITION AND PELTATE GLANDULAR TRICHOME NUMBER ON *Mentha piperita* PLANTS?**

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Poster / Plants-organisms interactions

Plant responses to stress are manifested at organ, tissue or cellular level, and modify the biochemical, physiological and developmental processes including essential oil (EO) biosynthesis and glandular trichome (GT) development. Inoculation with plant growth-promoting rhizobacteria (PGPR) has gained considerable attention because of their capacity to increase EOs yield and to ameliorate the effects of stress. The aim of this work was to determine the effect of inoculation with PGPR on *Mentha piperita* plants cultivated under drought stress over EOs biosynthesis and GT number. *M. piperita* shoots were inoculated with PGPR strains *Bacillus amyloliquefaciens* GB03 or *Pseudomonas simiae* WCS417r. Drought stress was induced by the suppression of water (MS: moderate stress, SS: severe stress). The following nine experimental treatments were performed: (1) control: normal irrigation (100% field capacity); (2) moderate stress (MS): irrigation until 10 days before harvest, (50% field capacity); (3) severe stress (SS): irrigation until 20 days before harvest, (35% field capacity); (4) inoculated with WCS417 r; (5) MS+WCS417 r; (6) SS+WCS417 r; (7) inoculated with GB03; (8) MS+GB03; (9) SS+GB03. At 27 days after the inoculation, EOs yield and peltate GT density were evaluated. EOs yield increase 5 times in non-inoculated stressed plants (MS and SS) compared to control plants (non-stressed, non-inoculated), and the same trend was observed when plants were inoculated with GB03 or WCS417r. The plants with the treatments PGPR + Stress did not show differences with the correspondent control, only MS + GB03 showed a slight increase in EO yield in relation to MS non-inoculated plants. GT density increases in non-inoculated stressed plants and inoculated stressed plants. The inoculation did not show any effect of GT density in relation with non-inoculated stressed plants. The lack of significant differences between non-inoculated + Stressed and PGPR + Stressed plants suggest that stress or inoculation conditions per-se increase the analyzed parameters and there is not a positive interaction between them. We observed a relationship among the GT density and the EOs yield, this could be because these structures are responsible for the EOs biosynthesis and storage. However, from a biotechnological point of view, it could be considered the idea of managing drought stress through the inoculation with PGPR to improve the indirect production of EOs, since PGPR elicited mechanisms which contribute to plant health and increase the biomass among other parameters like the antioxidant status (data not shown, Chiappero *et al.*, 2019).

## THE EFFECT OF MAIZE DOMESTICATION ON THE SUSCEPTIBILITY TO *Dalbulus maidis* ATTACK AND THE INCIDENCE OF MAIZE VIRAL AND STUNTING DISEASES

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Symposium / Anthropogenic impact in plant-insect communication

Among the most important factors that affect maize production there are stunting and viral diseases, transmitted in a persistent propagative manner by leafhoppers and planthoppers. *Dalbulus maidis* (De Long) (Hemiptera: Cicadellidae) transmits the pathosystem known as “corn stunting disease” in which, corn stunt Spiroplasma is the most prevalent pathogen in subtropical areas of the Americas. *D. maidis* is a specialist insect that has a long history of coevolution with teosinte and maize. The incidence of corn stunting disease has increased in the north of Argentina in the last years, particularly since the introduction in the region of germplasms with temperate background and other agronomical practices such as the adoption of two sowing dates with “early” and “late” maize. *D. maidis* is attracted to its host by visual and olfactory cues and has a particular preference for germplasms with a predominance of temperate genetic background, widely adopted because of their higher yield. This preference is explained, at least in part, by the volatile organic compounds emitted constitutively by these germplasms. Also, the more domesticated, the more beneficial to the vector of the disease, not only due to the loss of chemical defenses such as benzoxazinoids, but because of the loss of semiochemicals that mediate mechanisms of top-down control of the vector. The most important natural enemy of *D. maidis* is the egg parasitoid *Anagrus virlai Triapitsyn* (Hymenoptera: Mymaridae). This small wasp fails to find its host in more domesticated germplasms, where the release of induced plant volatiles is sometimes impaired, possibly making them more vulnerable to the herbivores for their inability to “cry for help” in a tritrophic system.

## VIRTUAL ETHOGRAMS OF THE PRECOPULATORY BEHAVIOUR OF *Cydia pomonella* WITH A SYSTEMIC PERSPECTIVE THROUGH AGENT-BASED MODELS

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Poster / Insect communication

In general, most of the Tortricidae family species have been identified by their pest activity in interaction with several plant species. The codling moth (*Cydia pomonella* L.) is one of them and is considered by their economic importance due to the damage they cause to various cultivars: apple, pear, etc. One of the elements considered for pest management programs for decision-making is the monitoring of populations based mainly on the study of their precopulatory behaviour, for adults, movements, acts, and postures related to chemicals. Females act as emitters by attracting males through a sex pheromone which is produced in a glandular structure including wing movements, and the extrusion of the male hair pencils. Once the receptor organism (males) locates females and stays 2 cm away from them, males become emitters whilst they release a chemical substance (not reported yet) that could be involved in sexual selection. In this case, the precopulatory behavior of *C. pomonella* adults was represented in an ethogram, based on an exhaustive literature review and modeling in silico through the computational agent-based model from a systemic perspective. The computational model was built in the NetLogo software and the results show: 1) the sexual call through the dynamics of the chemical message that begins with the female emission, 2) the male response with the search for female persuasion and 3) the copulation. The model represents behaviour in a general way, omitting movements and acts; the dynamics of the message over time was possible through parameters such as: diffusion rate, release probability, evaporation rate, and pheromone concentration. Finally, the use of mathematical models and computational tools made it possible to simulate movements of adults. The new term “dynamic-of-chemical-messages” is suggested as a product of interdisciplinarity, which intends to integrate a systemic view into the simulated ethograms of insects.

## TOXICOLOGICAL INTERACTIONS BETWEEN SPINOSAD AND ESSENTIAL OILS ON THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (DIPTERA: TEPHRITIDAE)

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Poster / Semiochemicals and pest management

*Ceratitis capitata* is considered one of the most harmful pest species among fruit flies of economic importance. Historically it was controlled with insecticides but these products have negative impacts, such as pest resistance development. The present study aims to evaluate the toxicological interactions between toxic essential oils (EOs) (*Baccharis spartioides*, *Eucalyptus cinerea* and *Schinus areira*) and the bioinsecticide Spinosad on adults of *C. capitata*, in order to decrease the concentration of active ingredient bioinsecticide and prevent resistance development. We dispensed 2 µl of each test solution on each fly (EOs doses 10, 25, 50 and 100 µg/insect; Spinosad doses 25, 50, 100, 200 and 400 ng/insect). According to these results we then prepared 4 formulations of mixtures (EO and Spinosad) considering the Loewe additivity model ( $\frac{1}{8}$ ,  $\frac{1}{6}$ ,  $\frac{1}{4}$  and  $\frac{1}{2}$ ). Mortality was recorded after 24, 48 and 72 h of treatment. We calculated lethal dose for 50% of mortality (LD<sub>50</sub>) and isobolograms were used to determine toxicological interactions between mixtures of EOs and Spinosad. The LD<sub>50</sub> at 48 h were: for *B. spartioides*: 62,48 and 34,76 µg/insect; for *E. cinerea*: 107,25 and 53,02 µg/insect; for *S. areira*: 51,25 and 38,11 µg/insect; and for Spinosad: 826,68 and 266,57 ng/insect; for females and males, respectively. Synergistic activity was found for *B. spartioides*-Spinosad on females and for *S. areira*-Spinosad on both sexes; whereas antagonistic activity was found for *B. spartioides*-Spinosad and *E. cinerea*-Spinosad on males. Finally, additive activity was found for *E. cinerea*-Spinosad on females. We highlighted that the  $\frac{1}{2}$  *S. areira*-Spinosad ratio registered higher mortality than the highest dose of the insecticide (400 ng) administered alone. This mixture could be the basis for a new tool or for the integrated pest management of *C. capitata*.

## ANTS, AGRICULTURE, AND ANTIBIOTICS

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Plenary

The formation of beneficial symbiotic associations can be a form of evolutionary innovation: by establishing a symbiosis with a microbe, a host rapidly gains access to unique 'goods' and/or 'services' previously unavailable to them. Fungus-growing ants, including the charismatic leaf-cutters, are a textbook example of symbiosis. These ants cultivate specialized fungi for food. In exchange, the ants provide their cultivar fungus with substrate for growth, dispersal to new colonies, and protection from competitors. This ancient and obligate ant-fungus mutualism is host to specialized pathogenic fungi in the genus *Escovopsis* that parasitizes the ants' fungal gardens, thereby destroying their food source. To help overcome this garden pathogen, many fungus-growing ants associate with antibiotic-producing actinobacteria that help suppress the growth of *Escovopsis*. I will present our recent findings related to the use of antibiotic-producing bacterial symbionts in fungus-growing ants and our work that indicates that associations between antibiotic-producing symbiotic bacteria and insects is more widespread. I will discuss the use of symbiotic microbes of insects as a potentially vast and yet largely untapped source for the discovery of novel natural products.



## IDENTIFICATION OF SEMIOCHEMICALS IN *Ascia monuste orseis* (GODART, 1819) (LEPIDOPTERA: PIERIDAE)

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Poster / Identification and synthesis of semiochemicals

Kale caterpillar, *Ascia monuste orseis* (G.) (Lepidoptera: Pieridae), is considered an important pest for the botanical family *Brassicaceae* in Brazil and Argentina. Species of this family include varieties with nutritional and economical importance such as cabbage (*Brassica oleracea* (L.) var. *capitata*) and kale (*B. oleracea* (L.) var. *acephala*), the two most produced brassicas in Brazil. *A. monuste orseis* is controlled by insecticides from the groups of pyrethroids, carbamates, benzoylureas, neonicotinoids etc., which are inconvenient to the producer and consumer and can increase production costs. An efficient control method that has been gaining ground is behavioral control, based on the use of semiochemicals, which is an important component in Integrated Pest Management (IPM), for being safe and environmentally friendly products. The present work aimed to identify volatile compounds in *A. monuste orseis*. The compounds were obtained by dynamic headspace and solvent extraction. Samples were analyzed by GC/FID, GC/MS and GC/EAG. A male-specific compound of *A. monuste* has been identified as (E)-3,7-dimethyl-1,3,6-octatriene. In addition, 12 hydrocarbons present in ovipositor, clasper, forewing and hindwing extracts incited responses in the behavior test in electroantennography in both males and females. Seven of them have been identified as the linear alkanes heneicosane, docosan, tricosane, tetracosane, pentacosane, hexacosane and heptacosane, all of which have already been reported in Pieridae. Understanding how these compounds influence communication between individuals of the species can provide an important tool in the control of *A. monuste orseis*.

## STUDY OF POULTRY FEED AND ITS COMPONENTS FOR THE CONTROL OF *Alphitobius diaperinus*

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Poster / Semiochemicals and pest management

*Alphitobius diaperinus* (Coleoptera: Tenebrionidae) is a stored grain pest that is difficult to control and that causes great damage to poultry farming as it is a vector of pathogens and its presence affects the health and development of birds. Antecedents to the risks of poisoning and contamination of the environment, as well as a selection of pesticides resistant, other approaches to the control of *A. diaperinus* have been the target of studies such as the use of semiochemicals for the monitoring of insects-pests. The aim of the study was to investigate and identify the organic compounds released from the compound feed and the individual feed components of the poultry feed, in order to promote the prospecting of natural compounds with potential for application in the integrated management of this category in aviaries. The bioactivity of extracts extracted from the main space was evaluated in behavioral bioassays in a Y-shaped olfactometer, isolation and identification of bioactive compounds were performed by gas chromatography techniques coupled with flame ionization detector (GC-FID), gas chromatography coupled to electroantennography (GC-EAD) and gas chromatography coupled to mass spectrometry (GC-MS). The GC-EAD and GC-MS analysis of the extracted by dynamic headspace of the compound feed and of the individual feed componentes (corn and soybean grains and meat meal) allowed the identification of 4 bioactive compounds: nonanal, 4-ethylbenzaldehyde, 4-ethylacetophenone and geranylacetone. The compound feed extracts were more attractive to *A. diaperinus* than the individual feed componente extracts. These compounds were compared with synthetic standards and were tested individually and in mixtures, under the rules of 1, 10 and 100 ppm). *A. diaperinus* is implicitly attracted to nonanal, 4-ethylbenzaldehyde and 4-ethylacetophenone, and is repellent to geranylacetone.

## DIFFERENTIAL BEHAVIOR IN SPECIALIST WILLOW SAWFLY AND ITS EFFECT ON SECONDARY METABOLITES ON WILD AND DOMESTICATED *Salix* SPECIES

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Poster / Plants-organisms interactions

Plant domestication has resulted in the modification of specific plant traits to increase their yield and quality. However, selective breeding may lead to a reduction of chemical defenses, usually affecting parameters of herbivore behavior. Furthermore, domesticated plants can become more susceptible to damage by herbivore insects compared to their wild relatives. In Argentina, willow plantations (*Salix* spp) are important in forest production and can be attacked by the sawfly *Nematus oligospilus*. Here, we compared *N. oligospilus* preference and performance when it feeds either on the native and non-domesticated *S. humboldtiana*, or on the high domesticated *S. babylonica* (var. *Sacramenta*). We studied oviposition preference of *N. oligospilus* between both willow genotypes, either undamaged or after egg laying and larval feeding. We also compared the performance of larvae that initiated their development on the leaf, where eggs were laid, and measured secondary metabolites as willow response. Our results showed a marked preference for laying eggs on native *S. humboldtiana* regardless of prior insect damage. Emission profiles of volatile compounds varied between willow species and changed after larval feeding, especially in *S. babylonica*, which released nitrogenous compounds (an aldoxime and benzyl cyanide). Larval development time was shorter, and mass of larvae and pupae were lower when they fed on *S. humboldtiana* than on *S. babylonica*. However, constitutive and inducible salicinoids levels were higher on *S. humboldtiana* than on *S. babylonica*. Preliminary analysis of condensed tannins showed no differences between species. Our results showed that the higher preference of oviposition of this specialist sawfly on *S. humboldtiana* may be positively related with the larval tolerance to salicinoids, increasing mass of larvae and pupae. We suggest that the differential performance might be due to a lower nutritional value of *S. babylonica* rather than higher chemical defense levels, such as phenolic compounds.

## ENANTIOSELECTIVE SYNTHESIS OF METHYL-BRANCHED CUTICULAR HYDROCARBON (R)-(-)- AND (S)-(+)-3-METHYLHENICOSANE

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Oral / Identification and synthesis of semiochemicals

Methyl-branched hydrocarbons are organic compounds present in complex cuticular blend of many arthropods. Several functions are described for these compounds such as a waterproofing barrier and even in insect communication as contact pheromones, mediating behavioral and physiological responses. Furthermore, the presence of the methyl group in methyl-branched hydrocarbons allows for the formation of stereoisomers and therefore makes it interesting to identify the absolute configuration of methyl-branched hydrocarbons and the correlation with bioactivity. After the identification of 3-methylhenicosane (1) as a contact pheromone antagonist in fly communication, enantioselective synthesis for identification the absolute configuration of natural product and the bioactivity through bioassays were performed. Methyl (S)-(+)-3-hydroxy-2-methylpropionate was used as a chiral pool and carbon-carbon bonds were formed via a Wittig olefination followed by Pd-catalyzed hydrogenation and Cu-catalyzed  $sp^3$ - $sp^3$  cross-coupling. (R)-(+)- and (S)-(-)-2-methylhenicosanol (2) was the common intermediate in both synthesis and it was derivatized to the Mosher's ester and analyzed by NMR. (R)-(+)- and (S)-(-)-2 showed 97% and >99% enantiomeric excess, respectively. Methyl-branched hydrocarbon (R)-(-)-1 was obtained after nine steps in 11% total yield (>99% ee) and (S)-(+)-1, after seven steps in 43% (97% ee). Absolute configuration of the natural product present in fly communication will be determined through bioassays that will be performed using (R)-(-) and (S)-(+)-1.

## FALL ARMYWORM, IMPORTANT PEST OF MAIZE IN THE AMERICAS, CAN SUPPRESS HERBIVORE-INDUCED PLANT VOLATILE EMISSIONS

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Poster / Plants-organisms interactions

Fall armyworm (*Spodoptera frugiperda*) (Lepidoptera: Noctuidae) is an important pest in the Americas. The insect is particularly well adapted to feed on maize (*Zea mays*, Poaceae), and can tolerate and detoxify specific direct defence compounds. We studied whether the insect could also manipulate indirect defences in maize, and whether this would affect attraction of a common natural enemy. We compared the induction of volatile emissions by different noctuid caterpillars: fall armyworm, beet armyworm (*Spodoptera exigua*), Egyptian cotton leafworm (*Spodoptera littoralis*) and cotton bollworm (*Helicoverpa armigera*). To assess whether oral secretions played a role in the observed differences, we also compared the induction of volatile emissions by regurgitant of these species. In a six-arm olfactometer, we assessed whether maize plants induced by fall or beet armyworm affected attractiveness of the parasitoid wasp *Cotesia marginiventris* (Hymenoptera: Braconidae). Fall armyworm induced the release of lower amounts of herbivore-induced plant volatiles (HIPVs) than the other tested species. This may be due to compounds in the caterpillar's oral secretions, as plants treated with fall armyworm regurgitant released less HIPVs than plants treated with regurgitant of the other species. Interestingly, the observed HIPV suppression by fall armyworm did not affect the attraction of a parasitoid wasp. In conclusion, fall armyworm, a ferocious pest particularly well adapted to feed on maize, is able to repress volatile emissions in maize. However, this did not change the attractiveness of infested plants to an important natural enemy. Studying fall armyworm's ability to manipulate their host plants may contribute to the development of novel practices for pest management and control.

## A NEUROETHOLOGICAL APPROACH TO ODOR VALENCE IN FLIES ACROSS THE *DROSOPHILA* GENUS

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Poster / Molecular and neurobiological basis of chemoreception

Flies have an olfactory system capable of detecting hundreds of odors. Each detected odor has an innate odor valence and consequently, it triggers promotion or inhibition of different behaviors (e.g. oviposition, feeding, courtship, and locomotion). We are interested in how the innate odor valence is related to the ecological niche. In this work we studied odor identity and valence representation in the first brain olfactory center, the antennal lobe (AL), of five members of the *Drosophila* genus occupying different habitats. We focused on species with olfactory host choice-driven adaptations, in species of economic interests, and in the model organism *D. melanogaster*. First, we identified ecologically relevant odors using natural feeding substrates, and we performed a behavioral screen to determine the valence of each odorant. Our screen unravelled interesting cases of odour specialization as well as conserved olfactory perception in evolutionary related species. Second, we generated transgenic lines to study neuronal activity of olfactory sensory neurons (OSNs) in the AL using calcium imaging. We observed that the pattern of neuronal activation is vastly conserved in species closely related in their evolutionary history and niche occupancy, even in cases where individual odors elicited opposite behaviors, i.e. switch of odor valence. On the contrary, species that are very distantly related or that occupy strikingly different habitats show clearer differences in OSNs activity. Interestingly, in none of the cases did the change of innate valence correlate globally with a more dissimilar odor coding, compared to odors that evoked a conserved valence between species. These results suggest that changes in the primary detection of odors are neither the sole nor the most important mechanism underlying adaptive behavioral changes across *Drosophila* species.

**SMOKE OF *Capsicum baccatum* L. VAR. BACCATUM (SOLANACEAE) REPELS NYMPHS OF *Triatoma infestans* (KLUG) (HEMIPTERA: REDUVIIDAE)**

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Poster / Chemical ecology of vectors

Chemical control of the Chagas disease vector, *Triatoma infestans* (Klug) (Hemiptera: Reduviidae) with synthetic pesticides in Bolivia has become increasingly inefficient due to the development of resistance in the insects. In the Chaco region of Bolivia, guaraní populations have approached the problem by fumigating their houses with the smoke of native plants. Through interviews and field work with local guides, the main plant used by the guaraníes was collected and later identified as *Capsicum baccatum* L. var. *baccatum* (Solanaceae). The arena consisted of a glass cylinder containing two semicircles of filter paper in the base (one totally clean and the other half exposed to the smoke of burned plant tissue). Bioassays were performed with smoked filter paper pieces on days 0, 3, 6 and 9 in order to test for residual effects of the smoke. On each bioassay one fifth-instar nymph was placed at the center of the dish, where the movements of each nymph were recorded during 20 min. Twenty-five replicates were performed on each treatment. In dual choice bioassays, nymphs of *T. infestans* avoided filter papers exposed to the smoke of the plant until day 3. Chemical analysis of smoke and literature data suggested that capsaicinoids present in the smoke could be responsible for the observed effect. The data presented here provide a rationale for the use of *C. baccatum* var. *baccatum* to control the Chagas vector by the guaraní populations.

## DRAMATIC EFFECTS OF AN INVASIVE OUTBREAK SPECIES ON FITNESS OF A NATIVE HERBIVOROUS SPECIES IS INDIRECTLY MEDIATED BY PLANTS

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Oral / Plants-organisms interactions

Biodiversity of insects is threatened worldwide. Biological invasions are among the main drivers of species decline, frequently through direct competition of shared hosts or indirect impacts of pesticide treatment. Here we studied a poorly explored topic: Legacy effects of invasive herbivores that alter the chemical profile of plants to the detriment of later-visiting native insects. We evaluated the impact of foliar chemistry as altered by the early season invasive insect LD (*Lymantria dispar*) on performance of the mid-summer native insect AP (*Antheraea polyphemus*), using multiple genotypes of trembling aspen (*Populus tremuloides*). In a first experiment, 2 treatments were performed in late spring: treatment H, where 3<sup>rd</sup> instar LD were allowed to feed for 1 week on selected aspen branches and treatment C, control without feeding damage. After one month, in midsummer, AP neonates were deployed to all H and C selected branches. Larval survival and growth performance were evaluated after one month. In a second experiment, 5 aspen genotypes were selected at 2 different sites: site A= control trees, and site B= trees totally defoliated and reflushed after a LD outbreak. Individual leaves from trees at both sites were offered in the lab to individual L3 AP in Petri dishes in a no-choice design. Larval performance was evaluated until moulting to L4 or dead. Results show that AP survival and performance was strongly determined by aspen genotype and site treatment. High phenolic glycosides (PG) levels correlated with high mortality rates and poor AP performance. Legacy effects were not evident in the 1<sup>st</sup> experiment, after low levels of LD feeding damage. However, after the LD outbreak, PGs increased in reflushed leaves leading to dramatic impairment of native AP performance. Our findings shed light on unexplored impacts of invasive species on native herbivores and open a new area of research on legacy effects of biological invasions.



## SYNTHESIS OF PHEROMONE CANDIDATES OF *Telchin licus* (DRURY, 1773)

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Poster / Identification and synthesis of semiochemicals

The giant borer, *Telchin licus* (Lepidoptera: Castniidae), is a pest that causes great losses in sugarcane production in all regions where it is present, mainly in the Brazilian Northeast. Due to its endophytic habit, its chemical control is not effective, requiring large amounts of pesticides, in addition to hand-picking control, where the caterpillars are removed from the plantation. As the application of pesticides is hazardous, the use of pheromones has emerged as an alternative, due to its high selectivity and low toxicity. To use pheromones against this pest, the aim of this work is to carry out the synthesis of the isomers (Z,E)-2,13-octadecadien-1-ol and (E,Z)-2,13-octadecadien-1-ol, which are pheromone candidates of the species. From the retrosynthesis of these molecules, a synthetic route with 9 steps was proposed, common for both structures, with variations only in the reduction method. The route starts with the monobromination of 1,9-nonanediol, followed by the protection of the hydroxyl. After that, a carbon-carbon coupling with 1-hexyne was done, aiming to obtain unsaturation in the structure. Then, hydroxyl deprotection was performed, obtaining 10-pentadecin-1-ol. This was stereoselectively reduced using hydrogen and Lindlar catalyst, then brominated and a new coupling was made, this time with 2-(prop-2-in-1-yloxy)-tetrahydro-2H-pyran, forming (Z)-2-(octadec-13-en-2-yn-1-yloxy)tetrahydro-2H-pyran. This compound was submitted to an acid hydrolysis, obtaining the (Z)-octadec-13-en-2-in-1-ol, which was reduced with LiAlH<sub>4</sub>, forming the (E,Z)-2,13-octadecadien-1-ol. The compounds obtained were characterized by NMR, GC-MS and FT-IR. (E,Z)-2,13-octadecadien-1-ol was synthesized in an overall yield of 12.5%. 10-pentadecin-1-ol, a key-intermediate in the formation of (Z,E)-2,13-octadecadien-1-ol, was also synthesized. The route used made it possible to obtain the (E,Z) isomer, as well as an important intermediate in the formation of the (Z,E) isomer. It is expected to complete the route to form the (Z,E)-2,13-octadecadien-1-ol isomer, as well as increase the yields of formation of (E,Z)-2,13-octadecadien-1-ol.

## SEMIOCHEMICALS AND FLOWERING PLANTS TO INCREASE CONSERVATIVE BIOLOGICAL CONTROL

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Poster / Semiochemicals and pest management

The diversification of agroecosystems with flowering plants (companion plants) is known to increase biological control of pests by the provision of important resources (food and shelter) for the support of natural enemies' populations in cultivated fields. Semiochemicals, combined with companion plants, can also be used to increase conservative biological control in a strategy called attract and reward. However, volatiles emitted by companion plant flowers can also be attractive to parasitoids, with additive or synergistic effects to those of semiochemicals. The aim of the study was to analyze the effects of different flowering plants on soybean stink bug parasitoids. The attractiveness of flower volatiles from basil (*Ocimum basilicum*), buckwheat (*Fagopyrum esculentum*) and *Zinnia* sp. flowers on the parasitoid *Telenomus podisi* (Hymenoptera: Scelionidae) was studied in the laboratory with olfactometer bioassays. Field experiments were carried out in two stages. In the first stage, the effect of the plants on parasitoid recruitment and community composition and parasitism and predation indexes were evaluated. In the second stage, selected plants were studied in an attraction and reward system, including the sex pheromone of the Neotropical brown stink bug, *Euschistus heros* (Hemiptera: Pentatomidae) as an attractive semiochemical. In olfactometer bioassay, the plants showed an attractive effect on the egg parasitoid when contrasted against air. Basil and buckwheat were the most attractive plants in the laboratory and field. Higher parasitism and predation were observed in soybean plots with a buckwheat border.

## ENDOPHYTE-INDUCED GREEN LEAF VOLATILES IN GRASSES MODIFY THE GROWTH OF THE SYMBIOTIC FUNGUS OF LEAFCUTTER ANTS

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Poster / Plants-organisms interactions

Epichloë endophytes can increase the emission of the green leaf volatile (Z)-3-hexenyl acetate (Z3-HAC) by their host grass. Leaf-cutter ants can forage on grasses and change their foraging if collected leaves are unsuitable for their symbiotic fungus. Here we evaluated 1) whether endophyte presence in the grass modifies the foraging preference of leafcutter ants, and 2) If Z3-HAC per se can affect the growth of the ants' symbiotic fungus, *Leucoagaricus gongylophorus*. For the first experiment, we conducted a binary choice experiment with 5 laboratory ant colonies for 5 days. Each day, two different pots sown with *Lolium multiflorum* seeds, coming from the same population but with contrasting levels of endophyte infection (E+: 93% and E-: 5%), were offered to each ant colony that has never been exposed to the endophyte grass before. At the end of each day, we counted the proportion of tillers damaged in the E- and E+ pots. We found a significant effect of the interaction between infection level and time ( $p < 0.05$ ) suggesting that the foraging preference of leaf cutter ants diminish over time in E+ plants. For the second experiment, we sowed *L. gongylophorus*, extracted from laboratory colonies of *Acromyrmex ambiguus*, in Petri dishes with Malt Extract agar. In each plate, we placed an embedded cellulose filter paper with 2  $\mu\text{l}$  of mineral oil (MO) or 2  $\mu\text{l}$  of 1.42  $\mu\text{g}/\mu\text{L}$  Z3-HAC in MO (N=8). We evaluated fungus growth by measuring the area once a week for 6 weeks. Total fungus area did not change significantly between treatments, but a clear asymmetrical growth was observed in presence of Z3-HAC, where a greater fungus area was observed on the distal side of the volatile source ( $p < 0.05$ ). This significant impact on fungus growth suggests that Z3-HAC induced by symbiotic microorganisms could be a signal that mediates fungus – ant interaction.

## RECRUITING AND MAINTAINING A MULTIPARTITE SYMBIOTIC COMMUNITY THAT CAN DEFEND A BEETLE AGAINST FUNGAL PATHOGENS

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Symposium / Chemical Interactions Mediated by Microorganisms

Pathogenic fungi are major natural enemies of most insects, exerting significant selective pressures and regulating populations of these and other arthropods. Beyond a strong cuticle and immune defenses, associating with bacteria that are able to produce antifungals can be an effective strategy for insects to fight back in the evolutionary arms race against entomopathogenic fungi. However, recruiting and maintaining bacterial partners that are protective yet not harmful to the host, and can be reliably retained or regained every generation are key challenges in establishing a defensive symbiosis with bacteria. Phytophagous *Lagriinae* beetles are able to maintain a multipartite symbiotic community dominated by a bacterial strain with a reduced genome, but able to produce a protective antifungal polyketide. Despite the increased dependence to the insect host, this strain can be transmitted to plant hosts and its maintenance in the insect relies on the plant environment. Additionally, other low-abundance bacterial strains that co-colonize specialized symbiotic structures in the beetles are able to produce an array of antifungal, as well as antibacterial compounds that likely contribute to a broad-spectrum protection across different life stages of the beetle. Through bioassays, genomics, analytical chemistry and mass-spectrometry imaging we characterize the role of the members of the bacterial community in defense. Additionally, we are using genetic manipulation and infection assays to better understand the molecular factors regulating the establishment of the defensive symbionts in the insect host.

## **SAME SPECIES, DIFFERENT CHEMICAL PROFILING: GENOTYPE-ENVIRONMENT INTERACTION SEEKING BIOACTIVE MOLECULES**

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Symposium / Semiochemicals Identification and Synthesis

Despite significant advances in the production processes and agricultural controls, insects are still the main competitors of humans for food. As a result of breeding programs for productivity gains, among other characteristics, most agricultural cultivars currently available do not present efficient resistance mechanisms against insects. Therefore, the comparative metabolomic study between wild genotypes and cultivars can retrieve fundamental information on constitutive and induced resistance mechanisms, associating these results with plant defense genes, proteins, and secondary metabolites, opening a research field for the development of sustainable products and practices for pest control. Accordingly, this work aimed at metabolomic and proteomic studies of different soybean genotypes (*Glycine max L.*), evaluating their resistance mechanisms along with different biotic and abiotic stress factors. This study was carried out comparing biological and metabolomic data. The soybean genotypes were cultivated in a greenhouse under controlled conditions. Evaluating the induced resistance mechanisms, the genotypes were subjected to stress factors by drought, chemicals, and herbivory (*Spodoptera cosmioides*). Biological assays were performed evaluating antibiosis and antixenosis responses. The metabolomic and proteomic analysis was performed by overlaying chromatographic and spectrometric (UHPLC-qToF MS and Headspace-GC-MS/MS) data. The instrumental extraction and optimization methods were developed using chemometric tools. Combining biological and chemical data with multivariate (un)supervised statistical methodologies are contributing to reveal complex patterns of botanical defense metabolites, identifying resistant genotypes and bioactive molecules against pest insects.

## HEAD LICE RECOGNIZE AND PREFER HEAD ODOR OVER FOOT AND FOREARMS ODORS

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Poster / Insect communication

Human head lice, *Pediculus humanus capitis* De Geer (Phthiraptera: Pediculidae), are hematophagous parasites that infest human heads. They are extreme host specialists suggesting a strict selection behavior towards the human head by the parasites. Despite the public health relevance of *P. humanus capitis*, the role of chemical clues to select the human head is not well known. In the present study, we attempted to find out whether head lice recognize and select the odor of the head over the odor of other parts of the human body. Our results, using a two-choice olfactometer, demonstrated that head lice were highly attracted by the volatile compounds of the human odor air, but they did not show preference for the volatiles of head compared to those of other parts of the body (forearm or foot). Conversely, when head lice were exposed in an experimental arena to the whole human odor of different parts of the body, they showed a preferential response to the whole head odor compared with the whole foot or forearm odors. These results suggest that head lice can be oriented towards the human's head odor at a short distance of the host.

## HEAD LOUSE FECES: CHEMICAL AND BEHAVIOURAL ANALYSIS

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Poster / Insect communication

Human head lice *Pediculus humanus capitis* (De Geer) (Phthiraptera: Pediculidae) are insect parasites closely associated with humans, feeding on the blood of their hosts and causing them skin irritation and probable secondary infections. Despite being a severe nuisance, very few studies have reported on intraspecific chemical communication in head lice. Here, we evaluated the behavioral responses of head lice to the volatile compounds and solvent extracts from their feces. We also chemically analysed the main volatile components of these feces by CG-MS and those of the feces extracts by HPLC-MS. Head lice were attracted to the methanol extract of their feces but not to the hexane or dichloromethane extracts, suggesting the polar nature of bioactive chemicals present in head louse feces. Follow-up chemical identifications, in fact, showed the presence of hypoxanthine, uric acid, and another purine tentatively identified as either guanine or iso-guanine. Additionally, head lice were significantly attracted by volatiles emitted from samples containing feces. The volatiles emanated from feces alone contained 19 identified substances. The major compounds found were decanal, nonanal, hexanal, and acetic acid, together representing approximately 60% of the identified compounds. This work represents the first chemical evidence of intraspecies communication among head lice. The results support the existence of active substances present in the feces of *P. humanus capitis* that may be involved in its aggregation behaviour.

## MYCORRHIZAE AND THEIR PROTECTIVE ROLE AGAINST HERBIVORES IN A SOUTH AMERICAN WILLOW TREE

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Poster / Plants-organisms interactions

Arbuscular mycorrhizal fungi (AMF) interact with most terrestrial plants, altering their gene expression and secondary metabolism. So far, the role of this common symbiosis on the interaction between *Salix humboldtiana*, a willow native to the southern hemisphere, and its natural enemies has been ignored. Our objective was to evaluate how the level of colonization by AMF and herbivory by insects affects the production of secondary metabolites associated with defenses of *S. humboldtiana*. We carried out a factorial experiment in pots with cuttings of *S. humboldtiana* growing for 6 months with soil from a gallery forest of the Rolling Pampa. We had 2 factors: Addition of inoculum with 3 AMF species (I+: 7g inoculum, I-: sterilized inoculum); and Herbivory by generalist 4th instar larvae of *Spodoptera frugiperda* (H+: damage for 10 days, H-: without damage). We measured plant growth rate, emission of volatile organic compounds and we estimated the colonization by AMF in roots. We did not detect differences in the growth rate of the plants according to the treatments, while inoculation increased colonization of AMF by 40% in roots. The results showed that inoculation affected the volatile profile of plants with and without herbivory by increasing the total amount of compounds present. This change was more significant after damage by *S. frugiperda* for 10 days. Differential compounds were mainly mono and sesquiterpenes. These preliminary results suggest that soil microorganisms can play a role in the ability of *S. humboldtiana* to resist or tolerate its natural enemies.



## ROLE OF THE OXYLIPIN PATHWAY IN THE CONTRASTING BEHAVIOR OF SOYBEAN GENOTYPES TO BUGS ATTACK.

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Poster / Plants-organisms interactions

In plants, the oxylipin pathway is the main defense mechanism against herbivore attack, producing both direct and indirect defenses. In this pathway, lipoxygenases (LOXs) are the first enzymes to act. In soybean these enzymes participate in the regulation of chemical defenses against insect attack, such as protease inhibitors (PI). LOX and allene oxide synthase (AOS) enzymes regulate the metabolic pathway for the synthesis of the defense hormone jasmonic acid (JA) and defensive secondary metabolites, such as PI, isoflavonoids and terpenes. On the other hand, the enzyme hydroperoxide lyase (HPL) acts to produce compounds known as green leaf volatiles (GLV). These act as indirect defense molecules, and in some species stimulate insect feeding. To study the role of the oxylipin pathway in the defense of soybean against bugs, a field experiment was conducted in Londrina (Brazil), with two genotypes of contrasting response to herbivory (IAC-100 and WILLIAMS 82). Two treatments were carried out, with and without insecticide application, and the expression of the genes of the main enzymes of the pathway was studied. The results show that leaves of the resistant genotype (IAC-100) have an increased expression of LOX and AOS genes in the presence of bugs, and decreased expression of HPL genes both in the presence and absence of insects. This would suggest that the resistant genotype IAC-100 derives more resources to produce AJ-related defenses than to produce GLV, with respect to the susceptible genotype WILLIAMS 82.

## FINDING FRIENDS IN THE FOREST: VOLATILES OF BARK BEETLE-ASSOCIATED FUNGI HELP MAINTAIN SYMBIOSIS WITH BEETLES

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Plenary

Due to global warming, outbreaks of tree-killing bark beetles (Coleoptera: Scolytidae) are becoming more frequent and intense throughout the world. For successful attack, these pests rely on free-living fungal symbionts. Fungi of the Ascomycota that associate with bark beetles are known to contribute to beetle nutrition, immunity from pathogens, and protection against plant toxins. However, there has been little investigation about how this symbiosis is maintained since each generation of beetles and fungi must find each other anew. We previously discovered that fungi of *Endoconidiophora*, *Grosmannia* and *Ophiostoma* associated with the Eurasian spruce bark beetle (*Ips typographus*) produce a range of aliphatic and aromatic esters and alcohols as well as terpenes when growing on spruce bark-amended medium. Most recently, we found that some fungi can convert monoterpenes of spruce resin to oxidized products. Single-sensillum recordings from bark beetle antennae showed that beetles detect certain fungal volatiles and monoterpene metabolites, and possess olfactory sensory neurons specialized for these compounds. Blends of volatiles attracted beetles in olfactometer experiments, and beetles discriminated between symbionts and non-symbionts on the basis of their odor profiles. Our results suggest that fungal volatiles help beetles to choose their symbionts in host tree galleries, which they carry with them to their next host. Individual volatiles may provide information about the fungal species, its nutritional status and its ability to detoxify host tree defenses. In this way, fungal metabolites structure ecological niches not only for the fungi themselves, but also for their bark beetle symbionts.

## MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF ALDEHYDE OXIDASES FROM THE GREAT WAX MOTH, *Galleria mellonella* AS ODORANT DEGRADING ENZYMES

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Oral / Molecular and neurobiological basis of chemoreception

Odorant degrading enzymes (ODEs) are proposed to degrade/inactivate volatile organic compounds (VOCs) in millisecond scale time. Thus, ODEs play an important role in the insect olfactory system as a reset mechanism. Through transcriptomic approaches, several putative ODEs have been reported, but a few functionally characterized. It is likely that the inhibition of these enzymes could incapacitate the olfactory system and, consequently, disrupt chemical communication promoting and complementing integrated pest management. One main example is the beehive pest, *Galleria mellonella*, in which males produce the sex pheromone (nonanal and undecanal, as major components) for attracting females. Nevertheless, the aldehyde-based sex pheromone appears to be unsuitable for ethological control, due to chemical instability, short-range volatility and common presence in beehives. Therefore, the aim of this work is to characterize an odorant-degrading enzyme (ODE) that degrades the major sex pheromone components of *G. mellonella* at molecular and functional level. Putative sequences for AOXs were obtained from the comparison of transcriptomes. Likewise, to unravel the profile of volatiles that *G. mellonella* might be face besides the sex pheromone blend, VOCs were trapped from beehive and the identification was made by GC-MS. Moreover, wild *G. mellonella* larvae were reared and individualized in order to isolate total RNA from different tissues and both sexes. The expression of two AOXs was evaluated in different tissues and both sexes through semi-quantitative PCR. According to the results obtained from semi-quantitative PCR, tissue- and sex-based expression results showed that GmelAXO2 and GmelAOX3 were expressed in both sexes and they are not antennae-specific. Finally, 74 compounds were identified through GC-MS, such as terpenes, alcohols and acetate esters, however, 8 compounds were found to be of the aldehyde type. These compounds will be used to evaluate the activity of a recombinant GmelAOX expressed in *E. coli* (BL21).

## POTENTIAL USE OF SEMIOCHEMICALS AND VISUAL SIGNAL TO CONSERVATIVE BIOLOGICAL CONTROL OF STINK BUGS WITH EGG PARASITOIDS

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Poster / Insect Communication

The egg parasitoid *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae), is a biological control agent for stink bugs (Pentatomidae). Foraging behavior of this insect is affected by different stimuli. This work aimed to evaluate how semiochemicals and a visual stimulus affect the host search and selection behavior of *T. podisi* in the field. The experiment was conducted in three tests carried out in the years 2020 and 2021. In each test, 20 plots of 25 m<sup>2</sup> were delimited in a total area of 0.55 ha of soybean field. The semiochemicals were (*E,E*)- $\alpha$ -farnesene (AF) and methyl 2,6,10-trimethyltridecanoate (MT), both applied in the amount of 1 mg of compound/rubber septa, separately. The control consisted of septa impregnated with 100  $\mu$ l of *n*-hexane. The septa were placed in the center of each plot. The visual stimulus (VS) consisted of yellow nonwoven fabric ribbons (10 cm x 6 m each), attached to strings tied to stakes. The treatments were randomly distributed, with the AF, MT and VS being evaluated separately, as well as the combinations AF+VS and MT+VS and the total combination of treatments (MIX) (n= 3 for each treatment). Weekly parasitism indexes were estimated with sentinel eggs, parasitoid density with yellow sticky traps and stink bug density with the shake-cloth technique. The abundance of parasitoids and stink bugs was not affected by the treatments. However, the parasitism rates were higher in the plots with AF and MT separately. It is concluded that semiochemicals favor the parasitism and efficiency of *T. podisi* in the field, but no interaction with visual stimuli was detected.

## PREFERENCE OF THE CODLING MOTH (*Cydia pomonella* L.) THROUGH PHENOLOGICAL STAGES OF QUINCE (*Cydonia oblonga* MILL.) CULTIVARS

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Poster / Semiochemical and pest management

Codling moth (*Cydia pomonella* L.) (Lepidoptera: Tortricidae) is the major world pest of pome fruits. Quince (*Cydonia oblonga* Mill.) is the most important pome culture in San Juan province, Argentina. The oviposition by females and the choice of neonate larvae are key behaviors for the success of the species. These behaviors can be different among phenological stages and cultivars. By means of laboratory bioassays we studied the preference of gravid females and neonate larvae of codling moth, using dual-choice bioassays. This was assessed at four different phenological stages throughout the quince season. Four quince cultivars (Champion, INTA 37, INTA 117 and INTA 147) were compared by pairs, each pair was considered a combination. Time and final choice of neonate larvae were recorded (season 2019-2020). For oviposition preferences, the number of eggs laid on fruits and on adaxial and abaxial surfaces of the leaves, was recorded (season 2020-2021). For neonate larvae bioassay, significant differences were observed between phenological stages and among cultivars at development stage. INTA cultivars had the highest frequency of choice for larvae. Larvae preferred to reach the fruits, but a high frequency of wandering was observed. Significant differences were observed between phenological stages in oviposition bioassays mainly at the ripening stage along with a higher number of eggs on INTA 147 cultivar. The total number of eggs increased since the post-fruit set, being higher at growth and then declining at the ripening stage. In all cultivars the oviposition preference was observed towards the adaxial surface of the leaves. At ripening, a low number of eggs was observed in areas where fruit pubescence had been lost. Quince cultivar preferences of gravid females and choices of neonate larvae throughout quince season, along with preferred places for oviposition, are relevant to pest management and the chemical ecology of codling moth.

## THE CHEMICAL ECOLOGY OF INSECT PESTS IN URUGUAY, SIMILAR QUESTIONS IN DIVERSE STUDY SYSTEMS

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Plenary

Chemical ecology may involve using a model system to answer fundamental questions in biological interactions. It may also involve exploring chemically-mediated interactions in diverse study systems, focusing on both the signal chemistry and its ecological significance. This has been our approach; we have combined chemical and biological studies in various insect and insect-plant systems. A common purpose behind our approach has been to provide semiochemical-based tools for cleaner pest management strategies in our region. The first system I will discuss involves the characterization of sex-aggregation pheromones in *Disploschema rotundicolle*, a native longhorn beetle that attacks citrus plantations. Sex-aggregation pheromones in cerambycids show remarkable chemical parsimony, with ubiquitous short aliphatic 1,2-diols and  $\alpha$ -hydroxyketones. We successfully described the male-emitted pheromones in *D. rotundicolle*, finding the well-known conserved chemistry. However, our attempts to develop a mass trapping device have been so far elusive. During our field tests, synthetic cerambycid pheromones attracted untargeted native cerambycids that may themselves become new research targets. The second study system involves the tritrophic interaction among the invasive pest *Drosophila suzukii* (SWD), a host fruit, and the locally-established parasitic wasp *Trichopria anastrephae*. We evaluated fruit VOCs as potential host cues for the wasps. We found that SWD attack modifies fruit-VOC profiles, resulting in a differential behavioral response of *T. anastrephae* females. The third and most recent project does not involve an agricultural pest but the common honeybee, *Apis mellifera*. We are beginning to use chemical ecology tools to test sublethal effects of in-hive sanitary products. We are evaluating the effects of acaricides in the detection and learning of floral volatiles. We have found that EAG responses to a common floral volatile is influenced by the fumigant treatment with a potential acaricide. The implications on learning and on the actual capacity of using floral volatile cues are open for future work.

## USO DE SEMIOQUÍMICOS PARA EL CONTROL DE PLAGAS EN AMÉRICA LATINA DESDE LA PERSPECTIVA COMERCIAL

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Symposium / Semiochemical & Pest Management in Latam

Las últimas siete décadas han sido apasionantes en el mundo de la ecología química de insectos. Desde el punto de vista académico hemos pasado de descubrir los componentes químicos que median la comunicación entre insectos de manera intra e interespecífica, a elucidar los aspectos moleculares de como este sofisticado sistema ha llegado a constituir una de las principales razones del éxito evolutivo de los insectos. Por otro lado, desde un punto de vista comercial, las feromonas y kairomonas se han convertido en insumos para el manejo integrado de plagas insectiles. Compañías privadas en cooperación con investigadores de todo el mundo han unido esfuerzos para diseñar estrategias para el monitoreo y control de insectos plaga en los más diversos cultivos. Coleópteros como el picudo del plátano, *Cosmopolites sordidus* y el picudo de la palma (*Rhynchophorus palmarum*) se han convertido en ejemplos exitosos del manejo con feromonas de agregación y trampeo masivo de plagas que amenazaban seriamente la agricultura en América Latina. Otras plagas han representado retos dada la baja efectividad o inconveniencia del uso de feromonas, por lo que nuevas estrategias se han basado en el uso de kairomonas como es el caso de *thrips* y mosca del establo. Finalmente, un reto comercial y técnico es la especificidad de las feromonas y la universalidad de su uso, una situación ejemplificada por la especie *Spodoptera frugiperda*. Brevemente describiré y discutiré cada uno de estos ejemplos y los factores que han afectado su uso comercial en América Latina.

## INSECT INDUCIBLE METHANOL PRODUCTION IMPARTS THE BROAD SPECTRUM RESISTANCE TO CHEWING AND SUCKING INSECTS.

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Poster / Plants-organisms interactions

Pectin methylesterases (PMEs) is a large multigene family and demethylesterify the cell-wall pectins, which leads to the methanol emission. There are two types of PMEs: Type-I and Type-II PMEs. Type-I PMEs comprises a PRO region, which shares similarities with the PME-inhibitor domain and keeps PME inactivated, and it cleaved by Subtilase (subtilisin like serine protease). Interestingly, reports have shown that *Withania somnifera* emits more methanol even in control conditions as compared to other *Solanaceae* species. However, a dramatic increase in methanol-emission has been reported due to cell wall damage in biotic stress conditions. Therefore, *W. somnifera* was selected as a source to find the most bioactive and early-inducible PME for inducing broad-spectrum insect resistance. Using available transcriptomic data, 70 PMEs and 64 Subtilase genes were identified in *W. somnifera*. For the selection of early-inducible and highly bioactive PME and Subtilase, RNAs were isolated from *Spodoptera litura* infested *W. somnifera* leaf at different time intervals i.e., 0 min, 30 min, 2 h, 6 h and 12 h. Quantitative expression analysis suggested that WsPME26, Type-I PME member, and WsSBT3 are the most active PME and Subtilase during *S. litura* infestation, respectively. We have cloned the putative WsPME26 under the control of constitutive and an inducible promoter and transformed *Nicotiana tabacum* plants. Transgenic plants comprising constitutive and inducible WsPME26 expression systems have shown 75-85% mortality against both the chewing (*S. litura* and *Helicoverpa armigera*) and sap sucking (aphid and whitefly) insect pest on the 4<sup>th</sup> day and 6<sup>th</sup> day, respectively. The structural interaction of WsSBT3 with WsPME26 showed that WsSBT3 cleaved the processing motif and activated the mature PME. We finally suggest that biotic stress induced WsPME26 and WsSBT3 and their interaction promotes chewing and sucking insect resistance through enhanced methanol emission.



## BRONZE BUG *Thaumastocoris peregrinus* AGGREGATION BEHAVIOR: PRELIMINARY STUDY OF CHEMICAL CUES ASSOCIATED

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Poster / Insect communication

Aggregation behavior is relevant on insects' life history. Particularly, cues that arise of individuals' activity (feces and footprints) trigger this behavior. *Thaumastocoris peregrinus*, known as bronze bug, is a phytophagous hemiptera that feeds on eucalyptus species. Field and laboratory observations show that *T. peregrinus* is a gregarious insect, with numerous adults and nymphs co-occurring on the same leaves. However, there are no surveys that study the cues associated with this behavior. Using dual choice trials and volatile organic compounds (VOCs) analysis, we started to study possible cues involved in *T. peregrinus* aggregations. For dual choice assays, we used an experimental arena with 3 continuous zones: choice zones of the same size (3 x 2 cm), and no decision zone (3 x 1 cm) between them. To obtain stimuli for trials, we exposed one leaf to 10 females, or 10 males leaving them fed, walked, and defecated for 48 h (attacked leaf). For treated trial we compared the attacked leaf (by females or males) against intact leaf. Also, we did control trials by testing intact leaf on both choice zones. Then each trial consisted of leaving a single individual (females or males) to wander around the arena for 10 min. We registered the time expended in both choice zones and compared them by a paired *t*-test. Additionally, we analyzed VOCs chemical profile of feces as possible chemical cues. Head-space of females' and males' feces were collected by SPME and then analyzed by GC-MS. Preliminary results showed that: 1) males spent double time on leaf attacked by males ( $t_{0.025; 10} = 2.48$ ;  $p = 0.03$ ); and 2) we did not find differences between feces chemical profiles of females and males. Although these are not conclusive results, this work is the beginning of studies to understand *T. peregrinus* gregarious behavior and its associated cues.

## AN OLD AND A NEW MODEL FOR INSECT OLFACTION - FLIES AND LOCUSTS

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Plenary

The vinegar fly is a long-standing model for studies of insect olfaction. By analyzing the genomes of 99 different species of vinegar flies and evaluating their chemical odor profiles and sexual behaviors, we could show that sex pheromones and the corresponding olfactory channels in the insect brain evolve rapidly and independently. Female flies are able to recognize conspecific males through their specific odor profiles. Interestingly, closely related species show distinct differences in odor profiles, which helps to prevent mating between different species. Males, in turn, chemically mark females during mating so that they become less attractive to other males. The migratory locust offers a less developed but highly interesting system in a hemimetabolous insect going through phase changes. We have recently developed new tools to study the unorthodox olfactory architecture and chemical ecology of these animals. Using chemical analysis of relevant odor sources and heterologous expression of receptors we have deorphanised more than 50 of these. We have also developed CRISPR-cas9-based tools to both delete and insert genes, thereby providing a unique vantage point to gain a deeper understanding of locust olfactory ecology and physiology.

## ARE THE LARVAE OF *Spodoptera cosmioides* ABLE TO DETECT VOLATILES ORGANIC COMPOUNDS FROM DIFFERENT HOST PLANT SPECIES?

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Poster / Plants-organisms interactions

The arrival of genetically modified soybean (*Glycine max*) cultivars with both insecticide action (Bt-toxins) and herbicide-tolerant traits have increased the production and yield of soybean in South America. *Spodoptera cosmioides* (Lepidoptera: Noctuidae) is a Bt-tolerant polyphagous species frequently associated with host plants from the *Amaranthus* genus with reported herbicide-resistant populations. The high capability to survive in Bt-soybean cultivars turned *S. cosmioides* into a serious pest. Our goal was to determine if *S. cosmioides* larvae are able to respond to volatile organic compounds (VOCs) released by two *Amaranthus* species, *A. hybridus* (native) and *A. palmeri* (exotic), and two commercial soybean varieties (one expressing the Cry1Ac protein and other non-Bt soybean, being both resistant to glyphosate). We used a dual-election air-stationary olfactometer, which consists of a glass tube (50 x 4 cm; length:diameter). Fourth and fifth-instar starved (24 h) larvae (35 per treatment) were allowed to crawl from the middle of the olfactometer to its ends. Larvae were reared on an artificial diet. The following treatments (emitted VOCs) were tested: Bt-soybean leaf vs. air (control); *A. hybridus* branch (containing from four to six leaves) vs. air (control); Bt-soybean vs. non-Bt soybean; *A. hybridus* vs. *A. palmeri*; non-Bt soybean vs. *A. palmeri*; Bt-soybean vs. *A. palmeri*; and Bt-soybean leaf with pods (reproductive stage) vs. *A. palmeri*. Larvae responded to VOCs from both host plants when *Amaranthus* and soybean species were compared against air. *Spodoptera cosmioides* did not show any preference between neither the soybean varieties nor the *Amaranthus* species. However, significantly more larvae moved to the arm containing *A. palmeri* when this was offered vs. the soybean varieties. Our findings demonstrated a potential host preference of *S. cosmioides* by *Amaranthus* species; therefore, the presence of these weeds should be considered when planning *S. cosmioides* management strategies in soybean.

## TRAPPING OF TRIATOMINES, CHAGAS DISEASE VECTORS, IN EXPERIMENTAL BOXES THAT MIMIC AN INSECT NATURAL HABITAT

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Symposium / Chemical ecology of vectors

*Triatoma infestans* is the main vector of Chagas disease, a neglected disease, in South America. The early detection of the vector is necessary within current vector control programs. We carried out assays aimed at developing an efficient tool to monitor the insects at low population densities. Thus, we tested an odor-baited trap (SPT, a sticky pitfall trap) in experimental boxes that mimic an insect natural habitat under semi-controlled laboratory conditions, using 10 four instar larvae of *T. infestans* in each box. Nine trials were carried out on different days. Three experimental boxes were set simultaneously during 14 hrs each night to test independently: 1) a SPT baited with a synthetic odor blend (test, T); 2) a SPT with a mouse (positive control, C+); 3) an empty SPT (negative control, C-). The synthetic odor lure consisted of three sachets of LDPE each containing a solution of a host odor: L(+) lactic acid, valeric acid and ammonia. Seven of the nine trials were recorded using an IR video camera. The percentage of insects captured was intermediate in T (Median, 30%) in comparison to C- (10%) and C+ (70%). The percentage of capture was statistically different between T/C- ( $p = 0.001$ ) and T/C+ ( $p < 0.0001$ ) using the Tukey method implemented in R. The video recordings of the first 30 minutes of the assay showed that the activity level of the insects was significantly higher in C+ and T than in C-. Overall, the percentage of insects captured among those that visited the trap was greater in C+ (71%) than in T (33%) and in C- (0%). These results show that the SPT baited with a synthetic odor blend is able to capture triatomines, and promises to be an effective tool with respect to the standard triatomine detection method for monitoring house infection.

## HERBIVORY OF THE STINK BUGS *Nezara viridula*, *Dichelops furcatus* AND *Piezodorus guildinii* INDUCES EMISSION OF VOLATILES ORGANICS COMPOUNDS IN SOYBEAN (*Glycine max*)

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Poster / Plants-organisms interactions

The stink bug complex formed by *Dichelops furcatus*, *Nezara viridula* and *Piezodorus guildinii* is one of the primary soybean pests that feed in developing seeds, causing massive economic losses in South America. In response to insect herbivory, soybean synthesizes and emits a blend of volatile organic compounds (VOCs) from their leaves, which act as host-location cues for natural enemies of stink bugs. This systemic induction of defenses starts with the synthesis of LOX enzymes in the seeds, which are key enzymes to jasmonic acid (JA) synthesis that regulates VOCs' synthesis. Although emitted VOCs from soybean grown in controlled environments have been thoroughly characterized, little is known about the emission of VOCs in field-grown soybean. In the present study, we studied VOCs emitted constitutively or induced in response to herbivory of *D. furcatus*, *N. viridula* and *P. guildinii* in field-grown soybeans. While undamaged plants did not emit VOCs, herbivory treatment increased emission of the sesquiterpene  $\alpha$ -farnesene. Although after herbivory of *N. viridula* plants emitted only two volatiles, *D. furcatus* and *P. guildinii* attack induced emission of five and eight VOCs, respectively, including the green leaf volatile (Z)-3-Hexenyl acetate. Additionally, we determined the expression of LOX genes (LOX-1, LOX-2, LOX-3) through qPCR in undamaged and stinkbug damaged seeds. Although we did not detect any change of gene expression in undamaged or mechanically damaged seeds, expression of LOX1 gene was up-regulated more than twenty folds after herbivory. This study suggests that field-grown soybeans recognize attacks from stink bug species and respond accordingly, emitting a dissimilar blend of VOCs.

## VOLATILES RELEASED BY CORN SEEDLINGS SHOWING SHADE AVOIDANCE FEATURES ARE SILENCED AFTER STINK BUG ATTACK

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Poster / Semiochemicals and pest management

The Neotropical stink bug *Dichelops furcatus* F. (Hemiptera: Pentatomidae) has recently become a new pest of corn (*Zea mays* L.) in Argentina and Brazil. This stink bug species feeds on corn seedlings reducing the number of plants per hectare and crop yield. Volatile organic compounds (VOCs) released by corn seedlings in response to *D. furcatus* attack could recruit stink bugs' natural enemies. Since plant-plant competition affects both plant growth and defenses, it is possible that VOCs released after herbivory can be adjusted to the photomorphogenesis changes. Corn plants perceive neighbor competition by phytochromes B1 (Phy B1) and B2 (Phy B2), and phytochrome B corn mutants show shade avoidance features in absence of plant-plant competition signals. In this study, we assessed VOCs emitted by seedlings of the wild type corn inbred line France 2 (WT) and its isogenic mutants lacking either Phy B1 or Phy B2 after *D. furcatus* damage. We collected and analyzed VOCs by using coupled gas chromatography – mass spectrometry (GC-MS). We observed that Phy B1 mutant seedlings constitutively emitted higher amounts of indole, methyl salicylate and total volatiles than both, WT and Phy B2 mutant seedlings ( $p < 0.05$ ; ANOVA). However, WT seedlings strongly responded to *D. furcatus* attack by releasing qualitatively and quantitatively more volatiles than mutant seedlings (E.g. (E)- $\beta$ -ocimene, copaene,  $\beta$ -sesquiphellandrene, 3-hexenyl acetate and linalool) ( $p < 0.05$ ; ANOVA). These results suggest that high intra-specific competition inhibits corn seedlings responses to *D. furcatus* attack by silencing VOCs emission.

## BENZOAZINOID DETOXIFICATION BY THE WESTERN CORN ROOTWORM

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Poster / Plants-organisms interactions

Highly adapted herbivores can hijack plant secondary metabolites for their own benefit. For instance, the western corn rootworm, *Diabrotica virgifera virgifera*, can detoxify and sequester maize benzoxazinoids for its own nutrition and defense against entomopathogenic nematodes. However, the detoxification mechanisms remain unclear. We investigated how the insect larvae detoxify MBOA (6-methoxy-2-benzoxazolinone), a benzoxazinoid breakdown product formed upon maize injury. We observed that MBOA is stabilized by the insect through N-glucosylation. By combining transcriptomic and metabolomic analyses, we identified 11 candidate genes encoding for N-glucosyltransferases in the insect gut. RNAi-mediated silencing of two of these candidates resulted in decreased levels of MBOA-Glc in the insect. Interestingly, the observed MBOA-Glc reduction in silenced larvae was not sufficient to significantly enhance the herbivore susceptibility to entomopathogenic nematodes. Discovering how a pest herbivore detoxifies benzoxazinoids and their derivatives may contribute to developing new pest management strategies.

## PHEROMONAL MODULATION OF BEHAVIOR IN ANTS

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Symposium / Emitters and receivers: Insights in insect communication and orientation

Considering the operational definition, pheromones typically elicit stereotyped and innate responses. In recent years, many studies on social insects, instead of focusing on the immediate and specific known response, have studied different behavioral responses that were found to be modulated by pheromones even when the pheromones are not present. As social insects, ants make extensive use of pheromones in a wide spectrum of behavioral contexts. For example, trail pheromones, typically involved in recruitment to a resource, trigger nest leaving and path following in recruited ants. Our goal was to study the modulation of behavior in ants after being exposed to the trail pheromone in two species of ants: the Argentine ant (*Linepithema humile*) and the carpenter ant (*Camponotus mus*). We wonder if trail pheromones can affect resource assessment, olfactory memories, and attention to sensorial cues for orientation. Taking into account that several species of ants are considered pests or are high-impact invasive species, the use of pheromones as disruptors of recruitment activity has been proposed as a highly specific and environmentally friendly control strategy. Therefore, studying the possible collateral effects that enhance or decrease control effectiveness could be relevant for the implementation of this strategy.



## QUEEN PHEROMONES ADVERTISE THE BENEFITS OF COOPERATION IN A FLEXIBLY SOCIAL BEE

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Oral / Insect communication

Cooperative groups rely on communication to allocate tasks, coordinate behaviors, and identify and respond to threats. The evolution of cooperative social behavior is linked with increased complexity and diversity of communication signals, but the origin of novel signals during this process is poorly understood. Pheromones emitted by the reproductive queens of eusocial insect societies enforce the reproductive division of labor characteristic of these groups by influencing the behavior and physiology of non-reproductive workers. These queen pheromones have evolved repeatedly across many independent origins of eusociality, but their early history and origins are obscure. Facultatively eusocial species provide a window into the earliest stages of sociochemical signal evolution because solitary reproductive behavior is co-expressed alongside eusociality, enabling direct comparison of chemical signaling between eusocial and solitary conspecifics. We pair these comparisons with behavioral bioassays to identify the methylalkane queen pheromones of a tropical halictid bee that exhibits intra-population facultative eusociality among foundresses, as well as flexible expression of the worker phenotype among their daughters. We further show that queen pheromone emission coincides precisely with the need for queen-worker signaling in social nests, and that these pheromones reliably signal queen ovarian development, egg-laying rates, and the fitness returns ultimately achieved by worker daughters. The amount of queen pheromone production by queens is also predictive of successful recruitment of daughters as non-reproductive workers. Together, our results support the hypothesis that queen pheromones originate as honest signals, rather than as chemical tools of worker control by queens. The use of honest queen pheromones by a facultatively eusocial insect provides strong empirical support for a model of eusocial evolution which predicts that these pheromones can facilitate evolutionary transitions from facultative to obligate eusociality.

## CHEMICAL ECOLOGY OF THE *Eucalyptus* BUG, *Thaumastocoris peregrinus*: 3-METHYL-2-BUTENYL BUTYRATE AS A POTENTIAL MANAGEMENT TOOL IN FOREST

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Poster / Semiochemicals and pest management

*Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae), a stink bug specialist in species of the genus *Eucalyptus*, has important implications in the forestry sector. Studies of its chemical communication have identified 3-methyl-2-butenyl butyrate, a pheromone produced in large quantities by males, to have a putative role in its sexual behavior. Previous laboratory tests have shown that the use of this pheromone, applied with rubber septa (10 mg), decreased oviposition by 10-20%. In the present work, we aim to optimize the use of this pheromone in disrupting the mating of *T. peregrinus*. Specifically, we tested different forms of dispensing it (sprinkling of solution, direct application on sheets, and dispensers with zip-lock bags), and analyzed their efficiency by detecting and quantifying the pheromone employing gas chromatography coupled to mass spectrometry (GC-MS) at different post-application times. From the modalities evaluated, the use of dispensers consisting of zip-lock bags provided the best results, allowing a gradual, sustained release over time. Using this dispensing modality, we evaluated mating disruption capacity by placing 15 pairs of unmated *T. peregrinus* adults in cages with bunches of *E. globulus* (1 leaf per pair). To determine the effect of the pheromone in the insect reproduction, we set up cages with a 3-methyl-2-butenyl butyrate solution (30 mg) or a control solvent (isopropanol), and recorded the number of total eggs per female at 5, 7, and 10 days. A 22% decrease in oviposition was observed for total eggs per female (GLM-Poisson:  $P < 0.001$ ), similar to the results obtained in tests with rubber septa. Overall, these results show the need to continue optimizing the use of this compound as a possible tool in the management of this pest species in the forestry sector.

## FORAGING PREFERENCES IN HONEYBEES AFTER EXPERIENCING ADULTERATED POLLEN

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Oral / Insect Communication

Deterrent substances present in food, like toxic and/or bitter compounds, can exert repelling responses in insects. Pollen, the main protein resource for *Apis mellifera* colonies, may present compounds that induce distasteful and/or malaise experiences in bees. Although honeybee colonies avoid collecting some low-quality pollens, evidence supports that foragers themselves are not able to make foraging decisions based on pollen composition at the food sources. We hypothesize that pollen assessment occurs after pollen is processed inside the nest, likely mediated by young bees, which might enable foragers to learn pollen cues and adjust their preferences to the most suitable pollens. To unveil the mechanisms that enable foragers to learn certain components of pollen, we performed dual-choice experiments with colonies confined in flying cages (9x3x2m). We compared foragers' preferences for two monofloral-pollen sources after one of them was adulterated with the deterrent amygdalin. The adulterated pollen was offered either: i) to foragers at the pollen source; ii) to all the bees inside the hive; or iii) to young bees transiently isolated from the colony during the treatment. Controls with unadulterated pollens were included. Interestingly, when the adulterated pollen was experienced directly at the food source (i), foragers did not avoid it, but they did after pollen had been incorporated inside the hive (ii). Experienced young bees alone could not modify responses of inexperienced foragers (iii). Altogether, results suggest that assessment of pollen composition requires the resource to be experienced inside the hive for honey bee foragers to adjust their preferences to the most suitable pollens.

## RNAi TECHNOLOGY AS A PEST CONTROL STRATEGY IN COTTON CULTURE

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Poster / Semiochemicals and pest management

The biggest challenges faced in the cotton crop is pest control, this crop is highly affected by different orders of pest insects, causing large-scale agricultural losses. The main form of pest control used in cotton cultivation historically is through the use of insecticides, however, their use causes disadvantages. After the advent of molecular methods, new strategies for the control of these insects emerged, one of which is the RNA interference (RNAi) technique, which aims to silence genes essential for the survival of the target pest. Our work aimed to evaluate the use of RNAi technology in pest management in cotton, highlighting the efficiency, delivery method and the challenges faced. For this purpose, a bibliographic survey was carried out in the Scopus, PubMed and Web of Science databases, using the following descriptors: "RNAi and control pest and cotton". We have seen an increase in the number of publications employing RNAi technology to control cotton pests, especially in the last 5 years. Our findings demonstrate promising results regarding the efficiency of the technique; we find many examples for delivery methods of this technology such as artificial diets, nanoparticles, viruses or bacteria and microinjection. Finally, we think that there is no ideal model for the delivery of RNAi technology, instead the most suitable system will depend mainly on the characteristics of each target insect in the culture.

## MATING-BASED REGULATION OF ODORANT-BINDING PROTEINS SUPPORT THE INVERSE SEXUAL COMMUNICATION OF THE GREATER WAX MOTH, *Galleria mellonella* (LEPIDOPTERA: PYRALIDAE)

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Poster / Molecular and neurobiological basis of chemoreception

The greater wax moth, *Galleria mellonella*, is considered a pest that affects bee hives, causing a decrease in honey bee population. Usually, moths are controlled and/or monitored through sex pheromone-based traps, which increases pheromone concentration, disrupting sexual behavior of males. The greater wax moth, in particular, has developed an inverse sexual communication, in which males emit sexual pheromones to attract females. In view of this, the olfactory system is key to understanding this in order to guide future integrated pest management strategies. Thus, odorant binding proteins (OBPs), that are known to act as carriers of odorants in insect sensilla, appear to be crucial to understand this type of communication. Considering the above, the objective of this research was to identify the profile of OBPs and determine the expression patterns of these. Next generation sequencing (RNA-Seq) was used to identify OBP-related transcripts, which were later analyzed in different tissues of the moth by RT-PCR. Subsequently, the relative expression of the insects in virgin and post mating state was determined by qRT-PCR. Our results indicate that the greater wax moth has 20 OBPs distributed in different tissues, 17 of the 20 OBPs were significantly down-regulated after mating in females, whereas only OBP7 was up-regulated, as for males 18 OBPs were up-regulated after mating. Finally, these results support inverse sexual communication in *G. mellonella* and suggest an important role of OBPs in this mechanism.

## POSIBILIDADES DE INCORPORACIÓN DE LOS SEMIOQUÍMICOS EN EL MANEJO DE PLAGAS EN CULTIVOS DE ARGENTINA

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Symposium / Semiochemical & Pest Management in Latam

Desde la década del 90', las feromonas se han incorporado en el monitoreo y/o control de importantes plagas de cultivos extensivos e intensivos de Argentina. Esto ha ocurrido principalmente con plagas cuarentenarias como *Cydia pomonella* L., *Grapholita molesta* (Busk), *Anthonomus grandis* B. y *Lobesia botrana* (D&S). El estatus de plagas cuarentenarias ha dado un rol significativo al SENASA en la adopción de esta tecnología para los programas nacionales de control en los distintos sistemas productivos. En menor escala, el monitoreo con trampas cebadas con la feromona de *Pectinophora gossypiella* S. se usó para decidir su control con insecticidas en algodón. Desde el 2013 se cuenta con un sistema de trapeo masivo de *Tuta absoluta* M. para la protección del cultivo de tomate. *Lasioderma serricone* (F.), es monitoreada con feromonas durante la recepción del tabaco para su industrialización. También hay tecnología disponibles comercialmente para el monitoreo de plagas de granos almacenados. Actualmente, el mayor conocimiento de estas sustancias, la presencia de mayor número de empresas dedicadas a los semioquímicos a nivel comercial, nuevos grupos de investigación sobre la temática y la valoración de las ventajas en cuanto su eficacia, mínimos riesgos para el ambiente, los consumidores y usuarios; permiten considerar el control etológico como una alternativa viable técnica y económicamente en cultivos hortícolas: coles, tomate, pimiento, etc, frutícolas: kiwi, cítricos, etc. y extensivos como la soja, maíz, garbanzo y algodón. Estas posibilidades abarcan el monitoreo de adultos y las técnicas de control por trapeo masivo, atraer y matar y confusión sexual. Se considera entre estas posibilidades el uso de atraccidas formulados con atrayentes florales e insecticidas adulticidas. Dependiendo de cada situación, procesos de I+D+I, serán necesarios para contar con estos productos para ser incorporados a esquemas de MIP, con un imprescindible acompañamiento profesional, que permita obtener los mayores beneficios de esta tecnología.

## EVALUATION OF A SYNTHETIC HUMAN ODOUR BLEND FOR THE ATTRACTION OF THE SAND FLY *Lutzomyia longipalpis*

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Oral / Chemical ecology of vectors

The sand fly *Lutzomyia longipalpis* is the main vector of *Leishmania infantum*, the etiologic agent of visceral leishmaniasis in Latin America. Despite the epidemiological relevance of this species, little is known about which odours attract host-seeking females. The aim of this study was to evaluate the attraction of *Lu. longipalpis* to a blend of volatile organic compounds released by humans, and originally developed for host-seeking female mosquitoes. The laboratory trials were carried out in a wind tunnel. Groups of three females were assayed (n = 30) for attraction to serial decadic dilutions of the odour blend, using hexane as the solvent control. Field trials in northeastern Brazil were carried out in a region of low- sand fly density, with the same dilutions of the blend. The traps were set up according to a 4x4 latin square design and replicated 3 times. In both the wind tunnel and in the field trials, female sand flies were attracted and caught in a dose-dependent manner. The field data provide evidence that the blend can be used for monitoring *Lu. longipalpis*, even in areas with low-sand fly density. Further studies will be conducted in other areas.

## DOMESTICATED vs. NATIVE VEGETATION: VARIATION IN VOC EMISSIONS AMONG *Solanum* GENUS PLANTS

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Oral / Identification and synthesis of semiochemicals

Indirect defenses are resources that plants develop to deal with biotic stress factors and consist mainly of the production and release of volatile organic compounds (VOCs) to attract natural enemies of insect herbivores or to alert neighboring plants. However, plant domestication could affect natural defense processes, as the production of VOCs. To identify and quantify the VOCs emitted by cultivated and wild tomato plants, we worked with the commercial species *S. lycopersicum* (cv Moneymaker) and wild species from South America (*S. habrochaites*, *S. pennellii*, *S. peruvianum* and *S. palinacanthum*). Also, we characterized the foliar morphology, through a description of the leaves trichomes by scanning electron microscopy. The VOC collection technique was fine-tuned, allowing the capture and subsequent analysis and quantification by gas chromatography-mass spectrometry (GC-MS). Additionally, a diversity index (DI) was calculated to estimate the change in VOC blend composition. The wild tomato species presented the highest concentration of sesquiterpenes, with Zingiberene, gamma-Elementene and beta-Caryophyllene being the most frequent and abundant within this group. The cultivated species released mainly monoterpenes. Additionally, we evaluated the effect of red light (730 nm) environmental enrichment on VOC emissions and composition in one of the wild species (*S. habrochaites* LA2167) and on the commercial *S. lycopersicum*. Our results demonstrate that VOC emissions are less affected in cultivated ( $24.3 \pm 4.0$  vs  $20.7 \pm 5.5$  ng. $\mu\text{l}^{-1}$ ) than in wild species ( $121.0 \pm 60.2$  vs  $901.0 \pm 97.6$  ng. $\mu\text{l}^{-1}$ ) by far red light. Conversely, under far-red conditions cultivated species showed an increase in DI (0.02 vs 0.03) and the wild species showed a drop (0.04 vs 0.02). From type-VI gland trichome analyses, *S. habrochaites* showed higher density than *S. lycopersicum* ( $4859 \pm 228$ .cm<sup>-2</sup> vs.  $3544 \pm 228$ .cm<sup>-2</sup>) and a reduction under far red light ( $4217 \pm 157$ .cm<sup>-2</sup>), while *S. lycopersicum* was without changes. In conclusion, far red light can affect the blend of VOCs in cultivated tomato and the quantity in wild tomato plants and, consequently, a different chemical signal is released to the environment.



**(Z)-JASMONE AS A RESISTANCE INDUCER IN COWPEA BEAN PLANTS *Vigna unguiculata* (L.) Walp. (Fabaceae) FRONT OF THE BLACK APHID *Aphis craccivora* Koch 1854 (HEMIPTERA: APHIDIDAE)**

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Poster / Semiochemicals and pest management

Cowpea is an important and low-cost food source in developing countries. One of the limiting factors for its production is the black aphid, *Aphis craccivora* Koch 1854 (Hemiptera: Aphididae), responsible for the transmission of the mosaic virus (Cowpea aphid borne mosaic virus), CABMV. The tools used in the integrated management of this pest are the use of semiochemicals, resistant varieties and the use of resistance inducers. The objective of this work was to study the mechanisms of action of the resistance inducer (Z)-Jasmone (CJ) in the activation of defense pathways in cowpea plants. For this purpose, samples of bean varieties treated with CJ were subjected to infestation with black aphid. The VOCs of the cowpea genotypes Vita 7 (susceptible), Guariba (resistant) and Gurgueia (tolerant) were identified in the treatments control plants and plants treated with (Z)-Jasmone (PCJ), and plants treated with (Z)-Jasmone and aphid (CJP) using GC-EM, evaluated the attractiveness or repellency of the VOCs against the aphid and carried out a test to verify the antibiosis using different doses of CJ. The results showed a variation in the emission of VOCs in plants treated with PCJ and CJP. In the free choice test, the VOCs of control plants of the BR19 Gurgueia genotype were attractive to insects, while the VOCs released by these genotypes after infestation showed insect repellency in the bioassays performed. Guariba and Gurgueia plant extracts after application of CJ were significantly repellent to black aphid. In the no-choice preference tests, the three doses of CJ induced antibiosis-type resistance in plants of the Vita 7 (susceptible) genotype, with the dose 75 g/ha showing the lowest number of live insects when compared to the other doses and control plants. The use of CJ proved to be promising in inducing plant resistance against the black aphid.

## ROOT MUTUALISTIC FUNGI MODULATE PLANT-HERBIVORE INTERACTIONS AT MULTITROPHIC LEVEL

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Symposium / Chemical Interactions Mediated by Microorganisms

Trichoderma and arbuscular mycorrhizal (AM) fungi are widespread soil inhabitants that establish symbiotic interactions with the vast majority of terrestrial plants conferring positive effects on growth and fitness. Several studies indicate that they can affect plant-insect interactions, still the mechanisms involved remain obscure. By combining untargeted transcriptomics and metabolomics, with performance and behavioral studies we aim to uncover key traits driving the impact of Trichoderma and AM fungi on the interaction of tomato plants with the specialist herbivore *Manduca sexta*. We found that root interaction with both fungal symbionts influences the shoot metabolome and transcriptome, priming the plant for strong accumulation of different defense-related compounds. As consequence, root inoculation with the microbial symbionts strongly altered the dynamics of the plant-herbivore interaction by negatively affecting the growth and survival rates, pupation success and sex ratio. Moreover, the fungal symbionts affected the performance and host preference by the parasitic wasp *Cotesia congregata*, by affecting the blend of volatiles released by the plants after herbivory, indicating that the effect of root symbionts on herbivores can escalate up to further trophic levels.

## CHEMICAL ECOLOGY OF SIREX NOCTILIO AND ITS SYMBIOTIC FUNGUS *Amylostereum areolatum* GROWN ON DIFFERENT SUBSTRATES

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Poster / Semiochemicals and pest management

The woodwasp, *Sirex noctilio* (Fabricius) (Hymenoptera: Siricidae), is an invasive species that has colonized most of the regions of the world where *Pinus* spp. is grown/cultivated. Since its establishment in Argentina in the 1980s, the wasp has become one of the main insect pests affecting the forestry industry. To date, an environmentally sound species-specific monitoring and control tool based on semiochemicals is lacking for this pest. To this end, knowledge on the sensory ecology of *S. noctilio*, specifically in terms of the chemical volatiles involved in eliciting attractive responses, is much needed. Previous studies carried out in our laboratory have shown a strong attraction of *S. noctilio* females to the obligate symbiotic fungus *Amylostereum areolatum*. In this context, our objective is to evaluate the response of *S. noctilio* females to different volatile stimuli released by *A. areolatum* grown in different substrates: artificial agar-based culture medium and two of the pine species most widely cultivated in Patagonia (*Pinus contorta* and *Pinus ponderosa*). Through 4-way olfactometric bioassays, we were able to determine that there is a clear hierarchy in relation to the preferences of the females towards the different stimuli evaluated, being the semiochemicals released by the fungus cultivated in *P. contorta* the most important. GC-EAD and GC-MS studies were performed to analyze the compounds emitted by the different sources and address the antennal response of females. The results are discussed in the context of possible species-specific semiochemical-based monitoring and control tools, and the directions of proposed future research.

## **SALINITY AND SURFACE TEXTURE CUES GUIDING *Aedes aegypti* OVIPOSITION BEHAVIOUR**

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Symposium / Molecular & Neurobiological Bases of Chemoreception

Oviposition (egg-laying) decisions are critical for the success of an organism. This is especially true in mosquitoes which have fully aquatic larval and pupal stages. The yellow fever mosquito *Aedes aegypti* lays its eggs singly above the waterline, where they can remain viable for months, hatching only when submerged in water. In addition to properties of the water itself, such as salinity, we hypothesized that surface texture is also an important cue for gravid *Ae. aegypti* mosquitoes as they decide where to lay their eggs. In this work, we describe a series of novel behavioural assays that test the oviposition preference of *Ae. aegypti* to varying textures and present results demonstrating a robust and roughness-dependent preference for textured surfaces, and investigate the interaction of surface texture and chemical cues, such as salinity, in guiding oviposition decisions.

## EVOLUTION OF OLFACTORY RECEPTORS TUNED TO MUSTARD OILS IN HERBIVOROUS DROSOPHILIDAE

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Oral / Plants-organisms interactions

The diversity of herbivorous insects is attributed to their propensity to specialize on toxic plants. In an evolutionary twist, toxins betray the identity of their bearers when herbivores co-opt them as cues for host-plant finding, but the mechanisms underlying this process are poorly understood. We focused on *Scaptomyza flava*, an herbivorous drosophilid specialized on isothiocyanate (ITC)-producing (Brassicaceae) plants, and identified Or67b paralogs that were triplicated as mustard-specific herbivory evolved. Using heterologous systems for the expression of olfactory receptors, we found that *S. flava* Or67bs, but not homologs from microbe-feeding relatives, responded selectively to ITCs, each paralog detecting different ITC subsets. Consistent with this, *S. flava* was attracted to ITCs, as was *Drosophila melanogaster* expressing *S. flava* Or67b3 in the homologous Or67b olfactory circuit. Thus, our results show that plant toxins were likely co-opted as olfactory attractants through gene duplication and functional specialization (neofunctionalization and subfunctionalization) in drosophilid flies.

## PLANT LITTER VOLATILES FUNCTION AS SIGNALS FOR DECOMPOSER ORGANISMS IN AN OLD-GROWTH PATAGONIAN FOREST

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Poster / Plants-organisms interactions

Plants interact with soil organisms in a myriad of ways, and this interaction is a key factor in determining the formation of soil organic matter and nutrient availability in terrestrial ecosystems. In some old-growth temperate Patagonian forests, three southern beech species (*Nothofagus obliqua*, *N. nervosa* and *N. dombeyi*) co-exist. We hypothesized that the chemical and morphological characteristics of leaf litter input, over long periods of time, may generate conditions for a specialized decomposer community, for both microbial communities and soil macrofauna. Our objective was to explore the possible mechanisms responsible for specialized plant–soil interactions in the decomposer community. We collected volatiles of unsterilized leaf litter of the three southern beech species, and analyzed their composition using a GC-MS. Our results demonstrate that each species has a completely distinct footprint of volatile organic compounds (VOCs) with *N. nervosa* dominated by sesquiterpenes and *N. dombeyi* composed of aromatic derivatives and monoterpenes. A single volatile was shared for all three *Nothofagus* species, the sesquiterpene  $\alpha$ -agarofuran. The leaf litter showed significant differences in  $\alpha$ -agarofuran emissions, with *N. nervosa* emitting more than 2000 times more than *N. dombeyi* ( $p < 0.05$ ). The behavior of decomposer organisms on agarofuran was evaluated. Two choice bioassays with the terrestrial isopod *Porcellio scaber* showed a strong avoidance of  $\alpha$ -agarofuran volatiles ( $p < 0.0001$ ). Volatile  $\alpha$ -agarofuran emissions caused differential development of Patagonian fungi with neutral, stimulatory and inhibitory effects on growth and extracellular enzyme activity. These results suggest that there may be signals that attract or repel epigeous macrofauna, and suggest that volatile signals can potentially modulate the behaviour of the decomposer community, with consequences for plant-soil interactions in terrestrial ecosystems.

## USING COMPANION PLANTS IN MAIZE NEGATIVELY AFFECTS FALL ARMYWORM

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Poster / Semiochemicals and pest management

In Brazil, 34% of maize production is cultivated by family-led farms, with 90% of the harvest being used for human consumption. However, losses in maize yield on such farms due to herbivores is high, reaching 40% of the production. This study aimed to evaluate if companion plants affect the population level of fall armyworm larvae, *Spodoptera frugiperda*, in maize and can consequently decrease production losses due to herbivory. Companion plants that were evaluated in field conditions were done so as border crops: *Crotalaria spectabilis* (Fabaceae), *Cajanus cajan* (Fabaceae) and *Pennisetum purpureum* (Poaceae). Two field experiments were conducted in 2019 and 2021 with 3 and 4 replicates of each treatment, respectively, in small (5m x 5m) plots. The treatments were: 1) maize as a monocrop, 2) maize with *C. cajan*, 3) maize with *P. purpureum*, and 4) maize with *C. spectabilis*. The number of herbivores in each plot was evaluated weekly, and levels of parasitism was evaluated with stink bug and *S. frugiperda* sentinel eggs, and with yellow traps. The results showed a significant reduction in the number of *S. frugiperda* larvae in plots with *C. cajan* and *C. spectabilis* as the border crop, and a higher level of natural enemies (parasitoids, *Scelionidae*) in plots.

## CLICK CHEMISTRY: IDENTIFICATION OF SEX PHEROMONES FOR NORTH AMERICAN CLICK BEETLE SPECIES (*ELATERIDAE*)

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Symposium / Semiochemicals Identification and Synthesis

Click beetles (Coleoptera: Elateridae) are well known to the general public because of the defensive clicking mechanism by which they can spring into the air when threatened. What is less well known is that a number of species are or have the potential to be serious agricultural pests, particularly in root crops such as potatoes and sugar beets, but also in crops such as grains and corn. Damage is caused mainly by their larvae, known as wireworms. There are estimated to be >1000 click beetle species in North America, yet surprising little is known about their biology. Several years ago, we initiated a project to start identifying pheromones for click beetles, with the goal of exploiting these chemicals for monitoring or possibly control programs. This resulted in the first identifications of pheromones for any North American species, and since then, several additional pheromones have been identified. Here, we will review what is known about North American click beetle pheromones, describe the strategies that we have used to try and identify new pheromones, and describe the identification of several novel pheromones for additional species.



## AN ELECTROPHYSIOLOGICAL CHARACTERIZATION OF ANTENNAL GUSTATORY SENSILLA IN THE ANTS *Camponotus mus* AND *Linepithema humile*

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Poster / Molecular and neurobiological basis of chemoreception

Although nectivorous ants feed mainly on extrafloral nectar, some genera such as *Linepithema* and *Camponotus* exploit additional resources from the anthropized environments they inhabit. They thus must decide if they feed on food sources with very dissimilar characteristics. Recent studies from our laboratory show that these genera respond differently to salts and acids. We performed extracellular recordings of single gustatory sensilla located on the antennal tip of *Linepithema humile* and *Camponotus aethiops*. We characterized their electrophysiological responses to solutions of sucrose, NaCl, ascorbic acid, and mixtures of these substances. The results show that sensilla of *C. aethiops* are less sensitive to sucrose than sensilla of *L. humile*. In both species, we classified two types of sensilla. Type-1 sensilla respond essentially to NaCl and maintain their response upon stimulation with mixtures of sucrose (100 mM) and NaCl (10-1000 mM). Type-2 sensilla exhibit potentiated (additive) responses to sucrose and NaCl mixtures. Small amplitudes of action potentials (up to 1.2 mV) were observed in both sensillum types upon stimulation with sucrose and low concentrations of NaCl, possibly corresponding to the same neuron. In both types, stimulation with a high concentration of NaCl (1000 mM) triggers higher amplitude action potentials, thus recruiting a different neuron. In Type-2 sensilla, stimulation with a mixture of sucrose (100 mM) and NaCl (10-1000 mM), small-amplitude potentials decrease while high-amplitude ones increase, suggesting that increase in NaCl within the mixture results in inhibition of sucrose responses. In both species, responses to ascorbic acid (0.01-1mM) were low. Yet, adding this acid to a 100mM sucrose solution inhibits the neural responses to sugar in *C. aethiops* and, partially, in *L. humile*. These results provide information about antennal gustatory receptors in ants, which have remained unexplored. Characterizing the sense of taste in ants is crucial for developing specific and efficient pest-control strategies.

## TREE DEFENSES UNDER DROUGHT CONDITION TO AGGRESSIVE BARK BEETLES: *Ips typographus* L. AND *Ips duplicatus* Sahg. (CURCULIONIDAE: COLEOPTERA)

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Poster / Plants-organisms interactions

*Ips typographus* and *Ips duplicatus* are considered essential pest species of insect to be controlled in Europe. A pioneer male tree-killer finds the tree host (*Picea abies* (L.) Karst.) boring and constructs a mating nuptial chamber. With a fast life cycle, infestation starts and causes tree mortality. In the Czech Republic, around 23 million m<sup>3</sup> trees per year between 2015 to 2019 were affected. To track these attacks is very important under climate change, a critical issue under the Anthropocene epoch. Interestingly, *I. typographus* does not present a specific niche area attack on its host tree, while *I. duplicatus* is reported attacking predominantly on the top of the host tree, especially on unhealthy trees affected by drought and windstorms. Abiotic and biotic aspects are most substantially associated with climate change as stress conditions and infestation by pest species of insects. In addition, trees logs used as traps in the field have shown work only to monitor *I. typographus*. With this evidence, bioassays were constructed and used to compare the physiological condition and tree ability defenses under drought stress and the attack of these two species. Analyzing this evidence will help to clarify the tree feeding behavior niche of these insect species and correlate with physiological and tree defenses traits. Our preliminary results showed that *I. typographus* successful attacks (excavating tunnel through the bark) to the host tree were 4.23 times more than *I. duplicatus* under the influence of drought stress. However, this successful attack of *I. typographus* decreased 1.7 times when compared with trees without a condition of drought as stress. *I. duplicatus* did not show a difference in the level of attack between control or stressed trees. Both cases *I. typographus* predominated the successful attacks. Statistical analysis is underway to analyze this evidence.

## **REGULATION OF POLLEN AND NECTAR FORAGING IN HONEYBEES: CHANGES IN GUSTATORY PERCEPTION, LEARNING AND MEMORY IN BEES ARRIVING OR DEPARTING FROM FOOD SOURCES**

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Poster / Insect communication

Foraging task specialization in honeybees, where foragers are specialized in the collection of pollen or nectar, is linked to differences in bees' sensitivity to flower's rewards. In behavioral bioassays, the successive offering of increasing concentrations of sucrose solution showed that nectar foragers start responding at higher concentrations, then are less sensitive to sucrose than pollen foragers. Moreover, as pollen foragers are less demanding regarding sugar rewards, they also learn better when nutritional and non-nutritional compounds of pollen are used as reinforcement during associative learning. So far, differences in gustatory perception have been observed between nectar and pollen foragers returning to the hive but have not yet been studied in bees at the beginning of their foraging visit (i.e., highly motivated to forage). By means of the proboscis extension reflex (PER), an innate response elicited when sucrose solution contacts the antennae, we measured the gustatory sensitivity of foragers arriving or departing from pollen or sugar feeders. In addition, we olfactory conditioned pollen foragers to study differences in acquisition and retention of odor – sucrose associations vs. odor – sucrose + pollen associations, at the beginning and at the end of the visits. Interestingly, at arrivals, pollen foragers were less responsive (i.e., present lower gustatory sensitivity) than nectar foragers and performed better with the dual reward (sucrose + pollen) than with sucrose alone. As it was expected for departures, pollen foragers showed higher gustatory sensitivity than nectar foragers and performed similarly during conditioning with or without pollen reinforcement. Our results are consistent with the fact that low sucrose responsiveness at the beginning of the foraging visit would prevent pollen foragers from being attracted to nectar sources while enabling them to learn source-related cues reinforced with pollen.

## OVIPOSITION RESPONSES OF *Aedes aegypti* AND IDENTIFICATION OF VOLATILES FROM MOSQUITO-ASSOCIATED SYMBIOTIC BACTERIA

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Oral / Chemical ecology of vectors

Complex oviposition decisions allow females of the mosquito *Aedes aegypti* to select suitable sites for egg-laying to ensure that their progeny thrives. Gravid females have been demonstrated to transmit key microbial associates to breeding sites by impregnating the egg surface with symbionts while ovipositing. Furthermore, bacterial communities present in larval habitats have been shown to influence mosquito oviposition behavior. Therefore, our study aimed to evaluate the effect of volatiles released by *Klebsiella*, symbiotic bacteria highly associated with mosquitoes, in eliciting oviposition site selection. For this, we first conducted dual-choice behavioral assays and demonstrated that volatile compounds released by *Klebsiella* can promote the oviposition decisions of gravid females. Bacterial volatiles were sampled by headspace solid-phase microextraction (SPME) and subsequent combined gas chromatography and electroantennogram detection (GC-EAD) analysis revealed that the antennae of gravid females detected two compounds present in the *Klebsiella* headspace. These compounds were identified by gas chromatography and mass spectrometry (GC-MS) analysis, identifying 2-ethyl hexanol and 2,4-di-tert-butylphenol in the headspace of *Klebsiella*. A binary blend of these two compounds elicited a dose-dependent response in oviposition-site seeking mosquitoes. We propose that bacterial symbionts linked to egg-laying release volatile compounds that act as oviposition cues indicating the suitability of active breeding sites to conspecific females.

## IDENTIFICATION OF BIOACTIVE COMPOUNDS AGAINST *Aedes aegypti* SCREENED BY BIOASSAYS AND *in silico* ASSAYS

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Symposium / Chemical ecology & vectors

Among hematophagous, *Aedes aegypti*, transmitting several critical arboviruses globally, such as dengue, Zika and Chikungunya. To reduce vector borne diseases transmission the usage of repellents should be considered. However, synthetic repellents have been broadly used leading to resistant vector populations, highlighting the need for discovery of new repellent compounds. *In silico* strategies have helped in such discovery by successfully identifying new bioactive compounds from natural sources, and demonstrating its potential to explore new repellent compounds. Thus, the repellent effect of essential oils extracted from *Lippia thymoides*, *Lippia alba*, *Cymbopogon winterianus*, and *Eucalyptus globulus* leaves was evaluated. All essential oils tested here showed repellent activity against *A. aegypti* in repellency bioassays, obtaining protection rates above 70% at 3.75 mg/mL or higher concentrations. GC-MS identified 57 constituents, which were used in the ligand-based pharmacophore model to expose compounds with requirements for repellents that modulate mosquitoes behavior through *A. aegypti* odorant-binding protein 1. This model approach results suggested that repellent activity observed from *C. winterianus*, *L. alba* and *L. thymoides* essential oils' metabolites is related to Citronellal (QFIT = 26.77), Citronellol (QFIT = 11.29), Citronellol acetate (QFIT = 52.22) and Geranyl acetate (QFIT = 10.28). *E. globulus* essential oil's repellent activity was associated with Ledol (0.94%; QFIT = 41.95). Molecular docking was applied to understand the binding mode and affinity of the essential oils' data set at the protein binding site. According to molecular docking, Citronellol (ChemPLP = 60.98) and Geranyl acetate (ChemPLP = 60.55) were the best-classified compounds compared to the others, and can be considered potential candidates to be explored and develop new repellents. Thus, using a combined approach of bioassay and *in silico* evaluation, we propose Citronellal, Citronellol, Citronellol acetate, Geranyl acetate and Ledol as possibly responsible for the *A. aegypti* repellency herein observed.

## PLANT PREFERENCE AND FORAGING BEHAVIOR IN THE LEAF CUTTING ANT *Acromyrmex ambiguus*

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Poster / Plants-organisms interactions

Identifying sensory cues that enable insects to find host plants, and understanding the neurobiology behind plant selection, provide crucial information for the design of sustainable pest management. This project aims at finding the main cues attracting leaf cutter ants *Acromyrmex ambiguus*, a major insect native pest of cultivated exotic willows in commercial plantations at the Delta del Paraná region. Given the interest in recovering native ecosystems, native plants were used. We selected eight native plant species and measured acceptance or rejection in field and laboratory experiments. We established a hierarchy from the most to the least preferred plants based on individual and on group behavior. Then by video analysis of ant foraging dynamics in controlled indoor nests we identified sequential steps along the foraging behavior, indicating an initial decision to approach to the plant that depends on olfactory cues, and second step that depend on gustatory cues and affect leaf-cutting and carrying-to-the-nest. Results on the most and least preferred plant species: "Sen del Campo" and "Anacahuita", respectively supported this dynamic. Volatiles and leaf cuticular compounds that modulate selection and preference behavior are being analysed by gas chromatography. We found that "Sendel Campo" and "Anacahuita" volatile profiles show qualitative differential profiles that increased their differences by mechanical damage. The compounds in this profile will be individually evaluated for their incidence in attraction or rejection behavior. This information might be used to strategically reintroduce native plants in a way that will be beneficial for conservation of the environment and sustainable production by taking profit of the ants activity.

## **CLASSICAL OLFACTORY CONDITIONING PROMOTES LONG TERM MEMORY AND IMPROVES ODOR-CUED FLIGHT ORIENTATION IN THE SOUTH AMERICAN NATIVE BUMBLEBEE *Bombus Pauloensis***

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Poster / Insect communication

Bumblebees are one of the most important pollinators for agroecosystems around the world. The South American species *Bombus pauloensis* (Hymenoptera: Apidae) is a commercially reared pollinator increasingly managed in greenhouse crops and in some outdoor commercial plantations. Recently, a study has shown that it is possible to train this bumblebee under a proboscis extension response paradigm using rewarded odours. Based on this study, we examined the capacity of this bumblebee species to establish stable and long term memories under an olfactory classical conditioning procedure and its ability to evoke information that has been acquired in another and very different behavioural context. Our results show that *Bombus pauloensis* can recall olfactory memories for at least 48 hours after training and that it is able to transfer memories from a classical to an operant context. These results support the possibility that, as in other social insects, social learning events related to resource information could occur within the nest of this South American bumblebee, an aspect with implications on collective foraging and recruitment.

## THE IMPACT OF AIR POLLUTION ON POLLINATORS

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Symposium / Anthropogenic impact in plant-insect communication

Multiple reports document the staggering decline of insect populations across the world. While some anthropogenic factors such as pesticide and land use change have been well studied, other factors such as air and water pollution have not been quantitatively examined in the field. India contains 90% of the world's top 10 most polluted cities according to respirable suspended particulate matter (PM<sub>2.5</sub>; RSPM). Five years ago, while surveying insects in our home city of Bangalore, we observed a significantly reduced number of pollinators compared to rural areas outside of the city. This reduction did not correlate with pesticide use, presence of flowers, or other obvious factors. We thus collected wild honeybees (*Apis dorsata*) from different sites. Our first SEM analyses provided a shocking realization – bees from more urban areas were literally coated in RSPM -a major component of air pollution. We thus embarked on a multiyear field-to-lab study to quantitatively correlate the presence of this PM on ethology, circulatory physiology, and gene expression of the Giant Asian Honeybee, *A. dorsata*, a native species that produces more than 80% of the honey in India. Our results indicate that wild bees exposed to RSPM (PM<sub>10</sub>) exhibit significant differences in flower visitation behavior, heart rate, haemocyte levels, expression of genes related to stress and metabolism, and ultimately survival. We then confirmed these impacts by directly exposing lab-reared and age-matched *Drosophila melanogaster* to the same field sites. Importantly, these effects were not predictable from similar human studies. Our study emphasizes the urgent need for more assessment of wild systems to inform scientific and policy-making decisions concerning human and environmental ecosystem health as well as economic loss to pollinator dependent crops and food security in highly polluted and vulnerable regions such as India.



## THE EPIPHARYNGEAL ORGAN OF THE BLOOD FEEDER *Rhodnius prolixus*

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Poster / Molecular and neurobiological basis of chemoreception

The detection of nutritional and toxic food by the taste sense or gustatory system is a crucial stage to ensure survival. Once a blood-sucking insect reaches the vertebrate host skin, it bites and tastes the blood. Gustatory evaluation of chemicals present in the blood occurs in putative gustatory chemoreceptors situated in the pharynx. Only if the insect's requirements are fulfilled, they initiate feeding. Although food detection and evaluation constitutes a highly relevant instance, the underlying mechanisms in blood-sucking insects remain largely unknown. In the case of the Chagas disease vector, *Rhodnius prolixus*, the epipharyngeal organ (EO) is a candidate to detect food components. Therefore, in this work, we characterized the EO of the kissing bug *R. prolixus* to identify its role in food assessment. Eight taste sensilla situated on the EO in the first portion of the food canal are the potential sensors of the food components. We hypothesized that gustatory receptor neurons (GRNs) and the cognate molecular receptors, housed within these sensilla, detect the components of the blood. The incoming information ultimately reaches specific brain regions through the GRN afferents for processing and integration. To address our hypotheses, we characterized the EO through neuroanatomical, physiological, and genetic studies. We located the cell bodies of putative GRNs underneath the epipharyngeal sensilla. We recorded neuronal responses in the EO upon stimulation with NaCl and ATP. The gene repertoire of the EO, obtained through RNA-seq, included representatives of several gene families candidate to encode sensory receptors. Finally, the GRNs of the EO reached the subesophageal ganglion in the brain through the labral nerves. This work provides the first characterization of a pharyngeal taste organ in a hematophagous insect.

## EFFECT OF LEARNING ON OVIPOSITION PREFERENCE IN *Drosophila melanogaster*

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Poster / Plants-organisms interactions

The olfactory, gustatory and visual senses help insects to identify potential sites for foraging, mating, and oviposition. All these activities can potentially be altered by learning. The different signals (visual, gustatory, and olfactory) received for the flies are processed mainly in the mushroom body (MB) and the lateral horn (LH), which are also involved in decision-making and learning. There has been extensive literature on the fly's olfactory sense, the behavioral response to different odors, and the learning process where classical conditioning experiments are employed to understand the impacts of attractive and aversive stimuli in insects' behavior. While a lot is known about foraging behavior, it is unknown whether the oviposition preference of flies can be altered after learning? What factors could affect the learning process during oviposition? We hypothesize that *Drosophila melanogaster* prefers substrates that they have already oviposited on. In preliminary experiments, we used different fruit substrates to check for oviposition preference of naïve and experienced flies. We could already show that flies indeed change their oviposition preference upon learning. Therefore, during this project, we will investigate how *Drosophila*'s oviposition preference is affected by experience and which chemical cues detected by the fly might be involved in this process.

## THE CHEMISTRY HIDDEN IN THE BIOLOGICAL INTERACTIONS FOUND IN THE CITRUS HOST

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Symposium / Chemical Interactions Mediated by Microorganisms

Phytopathogens have developed a variety of specialized virulence strategies to facilitate colonization of plant tissue and successfully modulate the host's physiology, including the production of low molecular weight phytotoxins (natural products). Brazil is the world's largest citrus producer, however citriculture is susceptible to several diseases that cause significant losses to our economy. In our projects, we try to elucidate the biologically active constituents of the host-pathogen interaction and study them with respect to functionality in the interaction. Understanding host-pathogen interactions could lead to the development of new, specific, efficient and safer antifungal compounds.

## PLANT GROWTH-PROMOTING RHIZOBACTERIA INOCULATION ON SWEET BASIL REDUCES THE GROWTH PERFORMANCE AND NUTRITIONAL VALUE OF *Spodoptera frugiperda*

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Poster / Plants-organisms interactions

Plant growth-promoting rhizobacteria play an important role in plant-insect interaction, but the understanding of the extent of their impact on insects is still in the early stages of knowledge. Therefore, the present study aimed at evaluating the role of inoculation with *Bacillus amiloliquefaciens* GB03 on sweet basil (*Ocimum basilicum* L.) in the development and nutritional parameters of *Spodoptera frugiperda*. In addition, the feeding preferences on inoculated and non-inoculated plants were assessed. *S. frugiperda* larvae reared with inoculated sweet basil leaves had a strong negative effect on the development of the insect, resulting in lower larval and pupal weights, and a longer period for larval – adult development, as well as reducing adult emergence but not affecting the relative consumption rate (RCR) value, thereby showing no alteration of the palatability. Moreover, growth rate and lower nutritional indices, such as the efficiency of conversion of ingested food (ECI) and the efficiency of conversion of digested food (ECD), were reduced in larvae fed from treated plants. In the choice test, larvae avoided feeding from inoculated leaves. The occurrence of a large number of secondary metabolites in the inoculated sweet basil plants could have been the reason for the reduction of the plant nutritional rate and also for the food selection, since it has been reported previously that GB03 inoculated sweet basil increased the essential oil yield. Therefore, PGPR inoculation could be used as a growth promoter, making it a promising candidate for plant protection programs against insects in aromatic plant production.

## SYNTHESIS OF JASMONE AND DERIVATIVES BY PALLADIUM MEDIATED C-C COUPLING.

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Poster / Identification and synthesis of semiochemicals

Insect pests are responsible for significant damage to agricultural products intended for human foods and animal feeds. Phytohormones such as jasmonic acid and other related compounds of the jasmonoid family have a defensive role in plants against pests. That is why they are presented as an ecological alternative for the development of technologies based on Integrated Pest Management. In addition, jasmonoids of the cis-jasmone family are structurally related to pyrethroids, known insecticides for their low environmental impact. This work presents the synthesis of a jasmonoid library by variation of side chains on a cyclopentenone core. We have recently developed a concise strategy for the synthesis of jasmonoids and derivatives. This methodology employs a variant of the Stille reaction that involves the use of  $\pi$ -allylpalladium complexes to link an alkenyl side chain to a cyclopentenone core. Through this method, different jasmonoid analogs have been synthesized by varying the structure of the alkenylic side chain precursor. In this way, the natural products jasmone, its four-carbon side chain analog cinerone, and several synthetic jasmonoids like allylrethronone were obtained. The carboxylic side chain was introduced by a Michael addition reaction to obtain jasmonic acid derivatives. In addition, the natural compounds dihydrojasmone and dihydrocinerone were obtained by standard hydrogenation with palladium on carbon. A preliminary biological evaluation of the jasmonoids was carried out targeting high incidence soybean and wheat pests. Experiments on the defense effects induced by methyl jasmonate in many pests were performed. In addition, preliminary preference tests were also performed using soy leaves treated with a simplified analog of jasmonic acid synthesized in our laboratory. On the other hand, field tests were performed using methyl jasmonate to attract aphid parasitoids and predators. These studies delivered promising results regarding the effects on insect development when natural and synthetic jasmonoids are used.

## INTEGRATIVE METABOLOMICS ANALYSIS OF THE BRAZILIAN BIODIVERSITY

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Plenary

Living organisms have long been used as a source for different useful human products. Traditional medicines have always been a source for the cure of many diseases since antiquity. However, their rational use was possible only after the understanding of how the compounds present in plants had their activities proved. Then the search for new bioactive compounds had a huge development and as consequence, a number of new molecules with different spectra of activities were found. Numerous examples of bioactive natural products are known, however their discoveries have always been associated with the development of new analytical techniques. Recently metabolomics strategies based on mass spectrometry improved the dereplication process and also opened new perspectives for chemical biology investigations. Even though the search for new drugs from plants and other organisms is still exciting and attractive, chemistry has played a key role in the explanation of biological and biochemical observations, opening a number of opportunities in the area. Each single organism (plant, marine or terrestrial animals, microorganism, algae, among others) interacts with the ecosystem by different strategies including chemical signals and/or chemical defenses. Compounds involved in this process are usually secondary metabolites, than we can accept the concept that organism actions are governed by the flux of energy and information through an enormous number of molecules. Also, natural products can have unknown physiological functions that must be deeply understood in the future. In this talk we provide an integrative overview of mass spectrometry strategies helping integrative metabolomics analysis and imaging generation for the Brazilian Biodiversity.

## COCOA FLORAL ODORS IN ATTRACTING POLLINATOR FLIES OF THE GENUS *Forcipomyia*

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Poster / Plants-organisms interactions

Flies of the *Forcipomyia* genus are considered to pollinate cocoa flowers, needed mainly for self-incompatible varieties. However, natural pollination corresponds to 0.3% of fruit production and the presence of pollinators in the field is low due to habitat fragmentation. The objective of this study was identify flower compounds of the varieties self-compatible CCN51, self-incompatible TSH1188 and Forasteiro (common cocoa) with potential attractive to pollinating flies of the genus *Forcipomyia*. Flowers of CCN51, TSH1188 and Forasteiro were highlighted near the floral opening time and their volatiles were collected by dynamic headspace technique. In the GC-MS analyses, 11 compounds were identified for CCN51 and TSH1188, while the Forasteiro variety has only 10 of these compounds in lower concentrations. Preliminary MS analysis suggested these compounds as saturated and unsaturated straight chain hydrocarbons, which is unusual among floral odors. The major compounds were tridecane, pentadecane, (Z)-7-pentadecene and (Z)-8-heptadecene. The bioactivity of the flower extracts was assayed using olfactometer in a Y-tube for adults of *Forcipomyia* sp. [n=117]. The treatments were: (I) CCN51 vs. hexane [n=19]; (II) TSH1188 vs. hexane [n=16]; (III) Forasteiro vs. hexane [n=20]; (IV) CCN51 vs. TSH1188 [n=20]; (V) CCN51 vs. Forasteiro [n=22]; (VI) Forasteiro vs. TSH1188 [n=20]. Bioassays showed a preference of flies to Forasteiro extract, with responses in 70%, 54.5% and 60% in treatments (III), (V) and (IV), respectively. For treatments with CCN51 and TSH1188, the flies showed weak attraction to the natural floral odor. The responses of CCN51 and TSH1188 were 37%, 40% and 23% in treatments (I), (IV) and (V) and 25%, 5% and 15% for (II), (IV) and (VI), respectively. The findings suggests that compounds released by cocoa flowers can modify the behavior of pollinating flies. More studies are needed to analyze the recognition of chemical signals and validate the reliability of the responses.

## OZONE POLLUTION AFFECTS EACH STEP OF THE CHEMICAL COMMUNICATION BETWEEN PLANT AND POLLINATOR

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Plenary

Volatile organic compounds (VOCs) emitted by flowers are key signals for the attraction of pollinators. Since pre-industrial times, the concentration of air pollutants has vastly increased. Among them, ozone ( $O_3$ ), due to its strong oxidative potential, may have the capability to alter: a) the emission of VOCs by flowers, b) their lifetime in the atmosphere and c) their detection by pollinators. To test these hypotheses, we used two plant-pollinator interactions, emblematic of the Mediterranean basin, but highly contrasted in terms of specificity and cognitive abilities of their pollinators: the highly specialized interaction between *Ficus carica* and *Blastophaga psenes*, and the generalist interaction between *Lavandula angustifolia* and *Apis mellifera*. Here, under controlled conditions, we exposed separately the plants, the VOCs or the insects to a high, but realistic,  $O_3$  concentration during a short period. Ozone exposure resulted in a clear change in the blend of VOCs emitted by figs but not by lavender plants. Moreover, for both systems, the proportion of VOCs present in the atmosphere surrounding the plants is altered, due to their different reaction times with  $O_3$ . Finally, we found that the ability of both pollinators to detect and behaviourally respond to VOCs is affected when they are exposed to high  $O_3$  concentration. These results suggest that chemical communication between plants and pollinators could be impaired by currently experienced  $O_3$  peaks. These findings highlight the urgent need to consider air pollution when evaluating pollinator threats.



## THE EVOLUTION OF CHEMICAL COMMUNICATION AND SPECIATION IN ORCHID BEES

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Symposium / Emitters and receivers: Insights in insect communication and orientation

Insects rely more on chemical signals than on any other sensory modality to find, identify, and choose mates. Male orchid bees acquire chemical compounds from various environmental sources including orchid flowers, fungi and rotten vegetation, and store them in highly specialized pouches in the hind tibiae. They release the resulting perfume bouquet in elaborate courtship displays at perching sites where mating takes place. Because perfumes are intricately involved in mating behavior and species recognition, perfume communication is thought to function as a pre-mating reproductive barrier among co-occurring orchid bee species. In this unique chemical communication system, the sense of smell is crucial for both perfume acquisition by males and perfume detection by females. I will be presenting recent research in which we identify rapid evolutionary change in an olfactory gene implicated in scent collection and mating behavior in two recently diverged species of orchid bees.

## BEHAVIORAL MANIPULATION OF *Drosophila suzukii* BY YEAST VOLATILES FOR PEST CONTROL

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Oral / Semiochemicals and pest management

The invasive pest, *Drosophila suzukii* (Matsumura) attacks fresh soft-skinned fruit and several control measures are applied to mitigate yield and economic losses in global horticulture. Among those, broad-spectrum insecticides are still widely implemented and there is a need to reduce environmental risks and insecticide residues on fruits. Chemical cues emitted from microbes are known to attract *D. suzukii* and provide an avenue for behavioral manipulation and sustainable control. *Hanseniaspora uvarum* (Niehaus) is a yeast frequently found on ripe grape berries and associated with *D. suzukii*. Aiming to exploit these ecological associations, we first investigated *H. uvarum* attractiveness. We show that *H. uvarum* volatiles mediate strong attraction of *D. suzukii* adults, both in laboratory and field trials. Subsequently, we aimed at *H. uvarum* as a basis for manipulating the flies' host finding and feeding behavior. We hypothesized that *H. uvarum* in combination with insecticides will induce *D. suzukii* odor-driven attraction and improve insecticide efficacy. In laboratory tests, we demonstrate that the addition of *H. uvarum* enhanced the efficacy of insecticidal formulations against *D. suzukii* when applied on green leaves. Flies exposed to leaves treated with yeast-insecticide formulations showed higher mortality and laid a lower number of eggs compared to flies exposed to insecticide alone. Furthermore, we provide evidence that the addition of insecticides did not affect *D. suzukii* attraction to yeast. Finally, in vineyard trials, yeast-based spray application on foliage only reduced *D. suzukii* infestation similarly to conventional insecticide treatment of the whole plant. Moreover, through restricting spray application to foliage we could reduce the total amount of sprayed insecticide up to one-third and prevent contamination of fruit with insecticide residues. Overall, we show that attract-and-kill technology based on yeast improves insecticide effectiveness and does not compromise management of *D. suzukii* in comparison to conventional insecticide application.

## SYNTHESIS OF 13-METHYL-HEPTACOSANE, CANDIDATE PHEROMONE FROM *Atheloca subrufella* (HULST)

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Poster / Identification and synthesis of semiochemicals

*Atheloca subrufella* (Hulst) (Lepidoptera: Pyralidae) is considered one of the main pests in coconut crops, causing damage to new fruits in their larval form, which decreases the harvest. Control methods for this pest are ineffective since the larvae develop inside the fruit and, when adult, lodge inside the inflorescences. The need for efficient alternative methods of control, which do not harm the environment or contaminate products extracted from coconut, drives the study of semiochemicals in behavioral control, a very promising technique in integrated pest management. The present work aims to synthesize 13-methyl-heptacosane, a pheromone candidate from *A. subrufella* (Hulst), to be used in its population monitoring or control. For the synthesis of this molecule, a synthetic route comprising seven steps was proposed. Initially, the bromination of 1-dodecanol is carried out using hydrobromic acid with 98% yield. The resulting bromide was subjected to a Grignard reaction with acetaldehyde to generate 2-tetradecanol (65% yield). The alcohol obtained is then oxidized using PCC in dichloromethane, generating 2-tetradecanone (87%). At the same time, the bromination of 1-tetradecanol was carried out, generating 1-bromotetradecane. A second Grignard reaction was performed using 1-bromotetradecane and 2-tetradecanone to obtain 13-methyl-13-heptacosanol (26%). The alcohol was dehydrated using p-toluenesulfonic acid in THF (63%). The corresponding mixture of alkenes was subjected to hydrogenation catalysed by palladium in CaCO<sub>3</sub>, giving the 13-methyl-heptacosane an overall yield of 6%. The products of each reaction were characterized by GC-MS and <sup>1</sup>H and <sup>13</sup>C carbon NMR. As next steps, it is expected to confirm the structure through comparison with the insect extracts and increase the yields of reactions, especially the second Grignard reaction, so that it is possible to develop traps to control *A. subrufella*.

## ELECTROPHYSIOLOGICAL RESPONSES OF *Xanthogaleruca luteola* (MÜLLER) (COLEOPTERA: CHRYSOMELIDAE) TO VOLATILE ORGANIC COMPOUNDS FROM *Eucalyptus globulus* EXTRACT

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Poster / Plants-organisms interactions

The insect *Xanthogaleruca luteola* (Müller) is a monophagous pest species native to Europe, known in Chile as "vaquita del olmo" (Elm leaf beetle). Larvae and adults of this insect cause damage to elm trees by feeding on its leaves. Although this species only uses *Ulmus* spp as host plants to complete its development, some preliminary observations have shown that it is attracted to non-host plants such as *Eucalyptus globulus*. In order to evaluate the response of the antennae in *X. luteola* males and females to the compounds contained in the hexane extract of the *E. globulus*, a gas chromatographic-electroantennographic detection (GC-EAD) was conducted. It was observed that female specimens only respond towards ten compounds, which in decreasing order of concentration in the extract are; 1.8-Cyeneol, Aromadendrene,  $\alpha$ - Terpineol acetate,  $\alpha$ - Gurjunene,  $\alpha$ - Pinene, Viridiflorene,  $\alpha$  -Terpineol, Globulol, Alloaromadendrene and Terpin-4-ol. The results obtained in GC-EAD suggest that the insect *X. luteola* has a highly selective system, capable of distinguishing semiochemicals from non-host species, however, it is necessary to deepen the role of each of the allelochemical substances found.

## THE SENSORY BASES OF NECTAR-SEEKING BY MOSQUITOES

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Plenary

Mosquitoes are important vectors of disease and require sources of carbohydrates for reproduction and survival. Unlike host-related behaviors of mosquitoes, comparatively less is understood about the mechanisms involved in nectar-feeding decisions, or how this sensory information is processed in the mosquito brain. Here we show that *Aedes* spp. mosquitoes, including *Aedes aegypti*, are important visitors of various floral nectar sources, including the *Platanthera obtusata* orchid. The associations between the mosquito and sources of nectar are mediated by a combination of the plant's scent, operating as a far-field cue, and the flower's visual display that operates as a near-field cue. The nectar scents are enriched in attractive terpene and aliphatic compounds that elicit strong antennal (olfactory) responses. Recording and imaging the neuronal activity in the mosquito's primary olfactory center, the antennal lobe (AL), revealed that the aliphatic and terpene compounds each activate distinct regions of the antennal lobe. The combination of compounds in these complex odors elicits distinct patterns of activity in the brain, allowing mosquitoes to discriminate between different scents. Moreover, we found that attraction to the flower's visual display is gated by the presence of the scent – without the scent, the attraction to the visual display does not occur. Together these results demonstrate the importance of mosquitoes beyond operating as disease vectors and open the door towards understanding the sensory bases of mosquito nectar-seeking behaviors.

## FEEDING ON SOYBEAN (*Glycine max*) MODIFIES GUT MICROBIOME DIVERSITY OF SOUTHERN GREEN STINK BUG (*Nezara viridula*) AND IT COULD INCREASE TOLERANCE TO CHEMICAL DEFENSES

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Poster / Plants-organisms interactions

The southern green stink bug (*Nezara viridula*) is one of the main pests of soybean, which reduces crop yields and produces important economic losses worldwide. Stink bug attack induces chemical defenses in soybean that can reduce or avoid damage. However, some insects can evade or tolerate plants' chemical defenses by microbiome that colonizes their gut. To determine whether the gut microbiome participates in the interaction between stink bugs and soybean, we studied the gut bacteriome of *N. viridula* adults reared in laboratory conditions and collected from soybean crops. The V3-V4 regions of the 16S rRNA gene were sequenced and analyzed to characterize the gut bacteriome of stink bugs collected from soybean crops from central-eastern Argentina and from a crop from an experimental field site at University of Buenos Aires, where laboratory-reared stinkbugs were fed on field-grown soybean for 20 days. Bioinformatic analysis was performed with QIIME2 and statistical analyses were performed with RStudio. We generated 66 operational taxonomic units from 17 gut samples, and four bacterial genera were identified. Three of them belong to the *Enterobacteriaceae* family: *Yokenella*, *Pantoea* and *Serratia*. In addition, the genus *Neoasaia* that belongs to the *Acetobacteraceae* family was only present in field collected insects. Results showed that the diversity and abundance of the main genera of gut bacteria are different between stink bugs reared in the laboratory and those collected from the field. The identified bacteria could be involved in the processes of stink bug resistance to soybean chemical defenses. These results highlight the importance of understanding the insect-microbiome relationships, which may play a role in facilitating or limiting the attack of the stinkbug to the soybean crop.

## ELUCIDATING OLFACTORY PROCESSING AND PLASTICITY IN THE FLY BRAIN

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Symposium / Molecular & Neurobiological Bases of Chemoreception

Most animals rely on their olfactory system to accomplish behavioral tasks that guarantee their survival and reproduction. Since the odor space consists of an enormous, nearly infinite number of possible stimuli, olfactory systems require special strategies to perceive, identify and evaluate the highly diverse odor information from the environment. The vinegar fly *Drosophila melanogaster* represents a premier model system for studying olfactory processing mechanisms since it exhibits a stereotyped architecture which is similar to its mammalian counterpart, but is less complex and highly tractable as well as susceptible to genetic manipulations. By exploiting these genetic techniques and linking them to neurophysiological, molecular and behavioral methods, my group is dissecting the neural circuits that are involved in coding, processing and perception of odors. We identified and dissected the neuronal correlates to specific behavioral outputs resulting from the perception of odor mixtures, we demonstrated that the neural composition of every olfactory glomerulus is unique and correlated to its functional relevance, and we were able to show that higher brain centers decode the behavioral value of an odor. We are currently examining whether the olfactory circuitry is hardwired or can be modulated by associative learning. The talk will summarize our recent insights into coding strategies and plastic components of the olfactory circuitry of *Drosophila*.

## DECIPHERING THE FEEDING BEHAVIOR OF *Rhodnius prolixus*

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Poster / Chemical ecology of vectors

In the presence of a food source, insects make a decision: to eat or not. Taste is the ultimate sensory modality involved in resource quality assessment, resulting in acceptance or rejection. Biting and feeding decisions depend strongly on the assessment of the chemical qualities of the food source. *Rhodnius prolixus* is a blood-sucking insect, vector of Chagas Disease. Here, we characterized the feeding performance of *R. prolixus* to molecules that favor or prevent ingestion. To do this, we used electromyogram recordings to examine the pumping activity of the sucking muscles and the biting behavior while the insect fed on different solutions in an artificial feeder. We developed algorithms and used machine learning to automatically quantify the different steps of the feeding process. Two sequential phases were evident: 1- food sampling or probing and 2- ingestion. Probing is designated as the time insects sip and taste the solution performing irregular pumpings without engorgement. Next, true ingestion begins if insects decide to feed, producing regular pumpings. We showed that glucose or leucine or phenylalanine triggered ingestion whereas caffeine, quinine, garlic extract, methyl salicylate prevented feeding. Differences in the feeding performances between these two categories (*i.e.* appetitive vs. aversive) of gustatory stimuli were evident. Probing time and number of bites to aversive compounds increased compared to appetitive compounds (39.6 %, 60 %, respectively). The number of pumpings, the time spent pumping and the pumping frequencies were significantly reduced for aversive compounds (95.5%, 93% and 24.8%, respectively). In addition, the volume taken in each pump, and the total time spent in the feeder significantly decreased to aversive solutions (41% and 69.7%). Knowing what and how molecules drive feeding or avoidance acquires particular interest, especially if novel tools can be developed to reduce the biting rates of blood-feeding insects of medical relevance.



## OLD DRUG FOR HUMANS REUSED TO RAPID DEVELOPMENT OF AGRICULTURAL DEFENSIVES

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Poster / Plants-organisms interactions

Few new pesticides are being developed for insects and microorganisms because of the high cost of pesticide discovery and the necessary years of arduous research. A possible alternative would be the reuse of old drugs for humans to test in a disease other than that it was developed. The best chance to identify new uses for old drugs is by testing them in a broad range of assays that may identify new targets. In this context, we have worked looking for old bactericides used for humans with the potential to generate new ones for agriculture by sustainable use of biodiversity. An example is quinine alkaloid, an important antimalarial drug. However, today there are the most effective antimalarial drugs. Quinine is obtained in large amounts in Brazilian Cinchona plants, showing that it can be obtained economically in Brazil. The cationic drugs are prone to interact with membrane phospholipids. Several representative amphiphilic cationic quinine-derivatives have been synthesized and evaluated against *Staphylococcus aureus*. Therefore, the search for new agricultural defensives led us to prepare amphiphilic cationic quinine-complexes. Quinine showed activities against *Xanthomonas citri* subsp. *citri*, *in vitro*, thus it was selected to be complexed with inorganic ions to improve their activities and solubility. We prepared amphiphilic cationic quinine-complexes using copper nitrate. In the bioassay *in vitro* against *X. citri* subsp. *citri* the complex  $[\text{Cu}(\text{PPh}_3)_2(\text{quinina})]\text{NO}_3$  showed a better MIC than pure quinine, MIC 0.21 and 0.31  $\mu\text{M}$ , respectively. The minimum bactericidal concentration (MBC) was also determined, confirming its bactericidal activity (MBC 0.21  $\mu\text{M}$ ). The biofilm inhibitory was also determined, and this complex inhibited the biofilm at MIC 0.21  $\mu\text{M}$  in 90%. The results suggest that this complex can be useful for controlling *X. citri* subsp. *citri* *in vitro*, and *in vivo* assays are underway.

## INTERSPECIFIC INTERACTIONS MEDIATED BY VANILLA VOLATILES WITH BIOACTIVITY TO INSECT PEST *Montella sp.*

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Poster / Plants-organisms interactions

Insects of the genus *Montella sp.* (Coleoptera: Curculionidae) have already been reported in the literature as pests causing economic damage, as well as pollinators, an increase in population is observed during the flowering period of the vanilla crop *Vanilla planifolia* Jack ex Andr., 1808 (Orchidaceae: Vanilloideae). There is oviposition on the flowers, which favors the newly emerged larvae to feed on immature fruits. To better understand the ecological interaction between plant-insect and analyze which allelochemicals are involved in this interaction and their bioactive action, this research sought to verify the bioactivity and identify the volatile organic compounds that mediate in the interspecific plant-insect communication. The execution of the experiments started with the obtainment of full flower extracts, and extracts of petals and sepals removed from the inflorescence, these were analyzed in a Gas Chromatograph coupled to a Mass Spectrometer. The extracts were also used for bioassays with a Y-type olfactometer, testing the bioactivity of insects for the natural extracts and the main synthetic compound identified. The presence of 5 compounds was identified in the extracts of sepals and petals removed, namely: (E)- $\beta$ -ocimene, eucalyptol, limonene,  $\beta$ -myrcene and  $\beta$ -caryophyllene. Of these, only the first two were found in full flower extracts. The compound (E)- $\beta$ -ocimene was the major compound in all samples, its concentration for sepals and petals was 170.9  $\mu\text{g mg}^{-1}$  and complete flowers 3.89  $\mu\text{g mg}^{-1}$ . The behavioral bioassays showed that the natural extracts and the synthetic (E)- $\beta$ -ocimene were attractive to males and females of *Montella sp.*, that is (E)- $\beta$ -ocimene should act as a kairomone function. The results clarify that there is an chemical ecological function that characterizes the population increase in the period of flowering of the crop and provides subsidies for the use of flowers or (E)- $\beta$ -ocimene in lure traps to capture the insect pest in the vanilla growing area.

## SYNTHESIS AND IDENTIFICATION OF UNUSUAL TERPENOID ESTERS ISOLATED FROM THE ANDROCONIA OF *Heliconius erato*

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Symposium / Semiochemicals Identification and Synthesis

*Heliconius erato* is a butterfly species extensively studied, with many evolutionary aspects discussed in the literature, such as pollen feeding and long longevity. *H. erato* exhibits Müllerian mimicry with other *Heliconius* species. The diverse wing color patterns are used for signaling predators about their toxicity, conferred by cyanogenic compounds they release. The color of the individuals is also associated with mating stimuli, and once the butterflies look alike the use of pheromones is essential for species maintenance. The androconia present on the hindwings of males is responsible for releasing species-specific compounds used as semiochemicals that are not found in other regions of the wing or other glands. Therefore, this is a very interesting region to explore mating signaling in *Heliconius*. The androconia extracts of *H. erato* presented two compounds with high retention index and very similar mass spectra that suggested terpenoid structures. They are released in high concentration and, in general, were the only major compounds detected in the extracts. We were interested in identifying these compounds, once they are specific for *H. erato* and might play an important role in mating choice because they are only found in androconia. After combining 60 extracts of the species and purifying the compounds, we had enough material for obtaining good NMR spectra. Combining this analysis with HR-MS, GC-FTIR and microderivatizations allowed us to make structure proposals. After synthesis, we were able to prove the compounds to be (4E,8E,12E)-4,8,12-trimethyl-16-oxoheptadeca-4,8,12-trien-1-yl oleate and (4E,8E,12E)-4,8,12-trimethyl-16-oxoheptadeca-4,8,12-trien-1-yl stearate. Bioassays are on the way to evaluate the role of these compounds as semiochemicals in *H. erato*.

## SEMIOCHEMICALS OF *Coccidophagous* LADY BEETLES AFFECT THEIR BEHAVIOR AND DEVELOPMENT

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Symposium / Emitters and receivers: Insights in insect communication and orientation

Lady beetles are important biological control agents in many crops and they produce semiochemicals that mediate their interaction with conspecific and heterospecific individuals. *Cryptolaemus montrouzieri* and *Tenuisvalvae notata* (Coleoptera: Coccinellidae) prey upon mealybugs (Hemiptera: Pseudococcidae), and can be found simultaneously in the same agroecosystem. This could lead to mutual interference in mealybug predation and dispersion of predators through chemical volatiles and footprints left in the environment. Thus, we have identified the profiles of volatiles and footprints released by these lady beetles through GC-MS and investigated their effects on the development, walking, and predatory behavior of the lady beetles. First, 52 different aliphatic hydrocarbons have been identified in the footprints of these species. In addition to the differences between species, there was a qualitative and quantitative difference in hydrocarbons between the genders. For the volatiles, 27 compounds were common to *C. montrouzieri* and *T. notata*. Despite that, eight compounds were specific for *T. notata*, with hexanoic acid identified only in female extracts and 2,2-dimethyl pentanol only in males. In *C. montrouzieri* there were 14 specific compounds, with benzothiazole found only in males. Furthermore, methyl-9-oxononanoate was specific for males of both species. Behavioral bioassays indicated that footprints of conspecific individuals did not affect the walking behavior of the lady beetles; however, the footprints of heterospecific individuals affected the walking behavior of males and females lady beetles. Moreover, predation bioassays showed that volatiles of conspecific and heterospecific individuals increased the predation rate of *T. notata* and *C. montrouzieri*. Finally, there was no effect of volatiles on the developmental times of *T. notata*, but for *C. montrouzieri*, there was a 2-day delay in developmental times when they were exposed to volatiles of *T. notata*. Therefore, results suggest that the semiochemical volatiles and footprints of lady beetles affect the intra- and interspecific interactions. The differences in the chemical profiles can indicate that the behavior recognition may be related to specific stimuli of the lady beetles. In addition, recognition of chemical profiles of conspecific and heterospecific individuals could lead to dispersion of competitive species such as *T. notata* and *C. montrouzieri*, which could, in turn, affect the biological control of mealybugs. These results can contribute to the adequate management of the studied coccinellids, aiming at the control of agricultural pests.

## CHEMICAL RESPONSES OF TOBACCO PLANTS INDUCED BY VIBRATIONAL SIGNALS OF A GENERALIST HERBIVORE

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Oral / Plants-organisms interactions

Plants are able to sense their environment and respond appropriately to different stimuli. Vibrational signals (VS) are one of the most widespread yet understudied ways of communication between organisms. Recent research into the perception of VS by plants showed that they are ecologically meaningful signals involved in different interactions of plants with biotic and abiotic agents. We studied changes in the concentration of alkaloids in tobacco plants induced by VS produced by *Phthorimaea operculella* (Lepidoptera: Gelechiidae), a generalist caterpillar that naturally feeds on the plant. We measured the concentration of nicotine, nornicotine, anabasine and anatabine in four treatments applied to 11-week old tobacco plants: a) Co = undamaged plants, b) Eq = Playback equipment attached to the plant without VS, c) Ca = Plants attacked by *P. operculella* herbivory and d) PI = playback of VS of *P. operculella* feeding on tobacco. We found that nicotine, the most abundant alkaloid, increased more than 2.6 times in the Ca and PI treatments as compared with the Co and Eq treatments, which were similar between them. Nornicotine, anabasine and anatabine were mutually correlated and showed similar concentration patterns, being higher in the Eq treatment. Results are discussed in terms of the adaptive significance of plant responses to ecologically important VS stimuli.

## DIURNAL VARIATION OF ODOR-BASED HOST SEARCHING BEHAVIOR IN *Trichopria anastrephae*, A PUPAL PARASITOID OF *Drosophila suzukii*

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Poster /Semiochemicals and pest management

The Spotted Wing Drosophila (SWD), *Drosophila suzukii* (Diptera: *Drosophilidae*), has become a threat to small berry crops worldwide. The female pierces the epicarp of ripening fruit to lay her eggs, causing secondary damage by other insects or microorganisms. The developing larvae cause the fruit to lose integrity, resulting in significant yield and economic loss. SWD has attracted much attention in the past few years; it is prolific and versatile in host utilization, making its control challenging. Biocontrol by parasitoids arises as a promising tool to incorporate in Integrated Pest Management (IPM) programs for SWD. In our region, *Trichopria anastrephae* (Hymenoptera: Diapriidae), a Neotropical parasitoid of drosophilids, stands out as a key candidate that is already adapted to local conditions. Host-finding represents a crucial step in parasitoid life cycles, and host-associated volatile organic compounds (VOCs) often play a key role in this process. Among the several behavioral and ecological factors involved in host searching, understanding how biocontrol agents respond to chemical information throughout the daytime may result in improved biocontrol strategies. Here, we studied the diurnal patterns of VOC-mediated innate attraction of *T. anastrephae* to host-associated odors. Volatiles from SWD-infested blueberries were evaluated in dual-choice olfactometer tests throughout the photophase to evaluate the diurnal variation in the response of *T. anastrephae* females. The results show that *T. anastrephae* attraction to host-associated volatiles follows a diurnal pattern, decreasing during the central hours of the day. The female wasps chose and spent more time in the olfactometer arm with VOCs from SWD-infested fruit, and this preference was significantly accentuated towards the last hours of daylight. Understanding the diurnal patterns of host searching by parasitoids may bear potential implications in IPM. Among these, appropriate timing of incompatible management practices such as pesticide applications may result in less damage to the biocontrol agent.

## **PATHOGENIC FUNGUS MODULATES INSECT-PLANT INTERACTION FAVORING ITS INFECTION AND DISSEMINATION**

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Oral / Plants-organisms interactions

The interactions among plant-insect-microorganism in the environment reveal a myriad of symbiotic strategies. For instance, volatile organic compounds (VOCs) have played a decisive role in these interactions. The VOCs induced by microorganisms result in the increased attraction of vector insects to the host and, hence, increased pathogen dispersal. By contrast, opportunistic fungi do not depend on vectors. Herein, we establish a new role for the insect-fungus-sugarcane association. It has long been assumed that *Fusarium verticillioides* is an opportunistic fungus, where it takes advantage of the openings left by *Diatraea saccharalis*' attack to infect the plant. Recently it has been demonstrated that *D. saccharalis* larvae feed on fungus, which is vertically transmitted to their offspring, unveiling an unprecedented insect-microorganism interaction. Here we also addressed the chemical identification of plant VOCs induced by fungus infection; then, we conducted electroantennogram (EAG) and olfactory preference assays to test whether the fungus influences the olfactory-based behavior of *D. saccharalis* adults. Additionally, fungi transmission in sugarcane plants by *D. saccharalis* was investigated by tracking the plant-insect system using a mutant *F. verticillioides* (*Fv:DsRed*). In this work, we show that *D. saccharalis* female moths not carrying *F. verticillioides* prefer to lay their eggs on fungus-infected plants, while females carrying the *F. verticillioides* prefer to lay their eggs on non-infected (mock) plants. EAG signal amplitude of 1-octen-3-ol (a sugarcane VOC) increased from infected female moths. Interestingly, 1-octen-3-ol contents increased in fungus-infected plant VOCs. Remarkably, this observed influence of fungus on moth responses allied with the vertical transmission of *F. verticillioides* on *D. saccharalis*, evidence an intimate plant-insect-microorganism relationship. Our findings alter the current understanding of *F. verticillioides* infection in sugarcane, demonstrating that the fungus manipulates plant and insect to promote its infection and dissemination, forming mutualistic but inherently selfish alliances with plants and insects.

## POLLINATION SYSTEMS AND NECTAR REWARDS IN FOUR ANDEAN SPECIES OF *Salvia* (LAMIACEAE)

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Poster / Plants-organisms interactions

Floral nectar shows high variability in the sugar composition which could be associated with visits of specialist or generalist pollinators. Adaptation to the most effective pollinator is often conceived as an explanation of widespread convergence in nectar characteristics of flowers. Also, morphological diversification of flowers of *Salvia* (Lamiaceae) species has shown to be attractive traits to specific functional groups of pollinators. In this work, we have determined visiting rate and effectiveness of the main pollinators to four species of *Salvia*, as well as production and sugar composition of their nectar. The main pollinators to the *Salvia* populations were bees, hummingbirds, bee flies and syrphid flies. The exclusively insect-pollinated species *S. stachydifolia* and *S. personata* displayed lower nectar volumes and higher concentrations than mixed- or hummingbird-pollinated species *S. orbignaei* and *S. haenkei*, respectively, suggesting that nectar quality and quantity are attractive to the energy requirements and the nectar consumption mode of the effective pollinators. However, we recorded an unexpected variety of strategies in nectar composition among the four studied species. Sucrose was the dominant sugar in *S. orbignaei*, while glucose dominated in *S. stachydifolia* and *S. haenkei*. In the case of *S. personata*, nectar was glucose-rich and completely lacked fructose. Our results support that pollinators have foraging preferences among certain types of nectar floral offered by *Salvia* species.



## EFFECTS OF MAIZE GRAIN EPICUTICLE ON GRAIN-INSECT-FUNGUS INTERACTION

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Oral / Semiochemicals and pest management

In general, the study of the interaction of the maize grain with its main pests, *Sitophilus zeamais* and *Fusarium verticillioides*, is carried out independently, focused on the study of the binary grain-insect or grain-fungus interactions, without considering the dynamics joint of the three organisms. However, the results of biological interactions are only fully understood when considered in the multitrophic environment in which these species develop. The present work aimed to determine, in a silo tritrophic experimental model, using two-way olfactometers, the participation of the epicuticle of maize grains in the contamination of healthy grains, from the migration of *S. zeamais* and *F. verticillioides*. For this, 4 treatments were proposed: Bitrophic G-I (maize grains + *S. zeamais*), Bitrophic G-H (maize grains + *F. verticillioides*), Tritrophic (maize grains + *S. zeamais* + *F. verticillioides*) and double control (only maize grains). After 32 days of experimentation, it was observed that both the double control, the Bitrophic G-I and the Bitrophic G-H did not present fungal mycelium development, while in the Tritrophic treatment the development of mycelium and percentage of mycotoxins was higher at the end of the olfactometer where the grains conserved their epicuticle. This result is in agreement with the preference of *S. zeamais* for maize kernels with epicuticle, indicating that the insect would act as a vector of the fungal mycelium and promote its development. The results obtained in this work allow us to conclude that the chemical signaling exerted by the epicuticle of the maize grain in the interaction with *S. zeamais* presents a predominance in the Tritrophic grain-insect-fungus interaction, being, in this experimental system, above to the physical protection that the grain pericarp can provide.

## INDIRECT DEFENSE OF PEAR PLANTS: HERBIVORE-INDUCED PLANT VOLATILES AND ATTRACTION OF PREDATORY LACEWING LARVAE

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Poster / Semiochemicals and pest management

Plants have developed different defensive strategies when attacked by herbivores or pathogens. Among these strategies, some plant species modify their volatile organic compound (VOC) profiles in response to herbivory, emitting herbivore-induced plant volatiles (HIPVs) that attract predators or parasitoids of the attacker. These HIPVs act as synomones in tritrophic systems, protecting the plant against herbivory and serving as chemical cues for natural enemies to locate their insect prey. This defensive strategy offers a new tool for the implementation of conservation biological control strategies by attracting pest antagonists to crop areas. In this study, we first analyzed the HIPVs of pear plants (*Pyrus communis* var William's) in response to the attack of herbivores of two different feeding guilds, the sap feeder *Cacopsylla bidens* (Hemiptera: Psylloidea) and the leaf chewer *Argyrotaenia spheropa* (Lepidoptera: Tortricidae). Second, we evaluated the behavioral response of a common predator, the lacewing larva *Crysoperla externa* (Neuroptera: Chrysopidae), to the VOCs emitted by pear plants under attack of the different herbivores. GC-MS analyses of VOCs from undamaged and damaged pear plants showed different HIPV profiles depending on the attacking herbivore. Moreover, VOCs from attacked pear plants elicited a differential response when offered to *C. externa* lacewing larvae in Y-olfactometer tests. The predatory larvae were attracted to VOCs from plants damaged by either herbivore when tested against clean air. However, VOCs from plants damaged by *C. bidens* were preferred when tested against VOCs from plants damaged by *A. spheropa* ( $p = 0.01$ ). These results show that pear plants can modulate their HIPVs depending on the attacking herbivore, and that this modulated response can be used by a predator to locate their preferred prey in a complex ecosystem.

## MOLECULAR SIGNATURES OF SAND FLY COMMUNICATION

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Male phlebotomine sand flies from Brazil produce pheromones that attracts other males to leks (aggregation) and females to the lekking males (sex). In addition, males produce songs — an acoustic signal generated by vibrating their wings — during copulation. Fly populations across Brazil are shown to utilize a unique combination of these songs and pheromones to communicate. Despite the extensive studies in sand fly communication that have provided important insights into the evolutionary biology of signaling and reception, little is known of their genetic signatures. I will present the complete annotation of the chemoreceptor genome repertoire in the *Lutzomyia longipalpis* and *Phlebotomus papatasi*, the two major phlebotomine vectors in the New World and Old World, respectively. Chemoreceptors interact with chemicals in an organism's environment to elicit essential behaviors such as the identification of suitable mates and food sources, thus, they play important roles during adaptation and speciation. Odorant receptors (ORs), gustatory receptors (GRs) and ionotropic receptors (IRs) together detect and discriminate the chemical landscape. Comparison with other sequenced Diptera revealed a large and unique expansion where over 80% of the ~140 ORs belong to a single, taxonomically restricted clade. Further, an analysis of single nucleotide polymorphisms (SNPs) and gene copy number in the chemoreceptors from 63 *Lu. longipalpis* individuals — collected from four different locations in Brazil representing allopatric and sympatric populations and three sex-aggregation pheromone types (chemotypes) — revealed multiple populations. Our work provides genomic insights into the underlying behavioral evolution of sexual communication in the *L. longipalpis* species complex in Brazil and highlights the importance of accounting for the ongoing speciation in central and South American *Lutzomyia* that could have important implications for vectorial capacity.

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